THE BOOK WAS DRENCHED

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PREFACE

THIS book is intended primarily for students of Botany and Zoology whose curriculum includes the study of the cell, either as a preliminary to the study of Genetics or as a separate course. As a result of conducting such courses for senior students of Zoology in the University of Edinburgh, the author believes that there is a need for a short work on general Cytology. Although there are some excellent books on nuclear Cytology, the writer does not know of any modern textbook which deals adequately with the cytoplasmic structures of cells. The present book, therefore, aims at supplying a general work which may prove helpful to teachers of Biology in colleges and schools who, through pressure of work or lack of access to current biological journals, are unable to keep in touch with recent research in a rapidly expanding branch of science.

It will be seen that some chapters deal exclusively with animal cells, some exclusively with plant cells, and others, for example the chapters on mitosis and meiosis, are applicable to both animals and plants. While the chapters are arranged to form a continuous sequence, at the same time it is possible, if the course so demands, for the student to select the chapters which meet his own special needs. Similarly, the teacher may plan his course according to the time available.

Wherever possible, reference is made to reviews or summaries of present knowledge, and older papers are cited only in special cases. The many instructive reviews and summaries of modern work on chromosomes made it possible, in the chapters on nuclear Cytology, to limit severely references to original papers, but the lack of recent summaries on extra-nuclear Cytology demanded a lengthy list of references in the chapters devoted to gametogenesis and fertilization in animals, and to the Golgi material and mitochondria of the animal cell. These chapters have taken shape, therefore, as reviews and summaries of original research, but the treatment ensures that the student may grasp the essential features without referring to the works cited, and the advanced student of Cytology may be guided in further reading.

The author is conscious of the difficulty of encompassing in such a survey as the present the whole field of Cytology, and is aware that reference to much important work is of necessity omitted. He will be satisfied if the present book provides a groundwork of cytological knowledge as it exists today and a stimulus to further study.

It is a great pleasure to acknowledge help received in the preparation of this book. Mrs. H. H. Clark, M.Sc., Lecturer in Agricultural Botany at King's College, Newcastle-upon-Tyne, in the University of Durham, wrote the chapters on Plant Cytology (Chapters IV, IX, X and XI) and prepared that part of the Glossary which relates to plants. The photomicrographs used to illustrate the text were taken by Mr. R. J. Fant, Department of Zoology, University of Edinburgh. Figs. 17, 19, 21, 23, 24, 41, 43, 57, 60 and 67 were executed by Mr. I. J. Linn, B.Sc., Department of Zoology, University of Edinburgh. Figs. 30 and 34 were drawn by Dr. I. Zlotnik, Royal (Dick) Veterinary College, Edinburgh. The print from which fig. 13, b, and the slides from which figs. 15, 16 and 22, a, b and d were photographed, were lent by Dr. B. M. Slizynski, Institute of Animal Genetics, University of Edinburgh. The slide from which fig. 62 was prepared was lent by Dr. K. Chodnik, Department of Zoology, University of Edinburgh. The author is grateful to Dr. G. Pontecorvo, Department of Genetics, University of Glasgow, for reading the typescript of the greater part of the work, and for valuable criticisms and suggestions, particularly relating to the chapters on nuclear Cytology; to Professor James Small, D.Sc., Department of Botany, Queen's University, Belfast, for helpful advice regarding the botanical section, and to Dr. I. Zlotnik for suggestions regarding the chapters on the Golgi material and mitochondria.

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CHAPTER I

INTRODUCTION

Cytology is the branch of biology which is concerned with the morphology and functions of the cell as a whole and with the structure and activities of its components. According to the *Cell Theory*, first formulated by Schleiden and Schwann in 1838–39 and later elaborated by other workers, the bodies of all the higher animals and plants are composed of minute structural units or *cells*, or the products of such cells The term "cell" was first used in 1665 by Robert Hooke, who showed that cork consists of small spaces surrounded by definite walls. It was not until a very much later date that attention was focused on the contents of the cell. As the original term has been retained by cytologists it must be clearly understood that the living cell is not a hollow space, but is a mass of protoplasm surrounded by a membrane.

The view that the cell is a primary organic unit, and that the body of a multicellular organism is built up of such units and exists as the result of their reciprocal actions, was first formulated in the Cell Theory. The cell, it was stated, is the primary organic unit of both structure and function. The multicellular organism is an aggregate of individual units which have undergone physiological division of labour. The Cell Theory focused attention on the importance of the study of the minute structure of the tissues and opened up an immense field of research. There was, however, a tendency to interpret the theory too rigidly and to regard the cell as an independent and more or less isolated unit. It is true that every cell arises through the division of a pre-existing cell, that the cell exhibits within itself the vital activities characteristic of life, and that embryonic and other cells can be grown in culture media outside the animal body. It must be recognized, however, that the cell in its normal environment within the body is subjected to influences from its surroundings. It interacts with, and is dependent upon, cells in other tissues of the body. Its life is merged to a considerable degree with that of the organism as a whole. It is differentiated, along with neighbouring cells, to perform certain functions, but the carrying out of its activities depends not only upon its components but also upon the activities and normal functioning of the organism as a whole Histological investigations have shown that syncytia exist, and that many types of cells are connected by

protoplasmic bridges with their neighbours. The cell, however, may be considered as a morphological and functional unit of protoplasm, in the vast majority of cases containing a nucleus, but it is in no way isolated from its environment and in some cases may be connected morphologically with neighbouring units. The tissues of the multicellular organisms are ultimately derived from a single cell, the *ovum*. During development structural and functional differentiation takes place, and at the same time there is a process of integration, so that the products of cell division remain connected physiologically if not structurally.

The cell (figs. 1, 3 and 6) consists of living substance called *protoplasm* which may contain *inclusions*, formed as products of cellular activity. It almost always contains a nucleus which consists of protoplasm having a somewhat different chemical composition to that of the remainder of the cell. The cell is bounded on the outside by a thin layer or surface film of living substance known as the *plasma membrane*, and the nucleus is surrounded by a membrane of a similar nature—the *nuclear membrane*. The *protoplasm* of the nucleus is called the *nucleoplasm*, and that of the remainder of the cell the *cytoplasm*.

In multicellular organisms cells of a similar type are grouped together to form *tissues*, and different types of tissues combine to form *organs*. It follows, therefore, that within the body of an animal or plant there are considerable differences in the size and shape of the cells of the various tissues. Such differences can be strikingly demonstrated by contrasting unstriped muscle cells, or epithelial cells, with nerve cells from the spinal cord of a large mammal (fig. 1). As regards volume, the largest animal cells are the eggs of certain fish, reptiles and birds. It should be remembered, however, that the eggs of these animals contain relatively little active living protoplasm, but possess a large amount of nutritive material or yolk.

Cells of the same type vary somewhat in size but, within a certain range, cell size is a specific constant. It has been shown that animals of the same species, which differ in size, do not vary to a large extent in regard to the size of their cells, but that the number of the somatic cells varies greatly, being most numerous in the larger individuals. Cell shape varies considerably in different types of tissue. The nucleus is usually rounded, and in general shows little correlation with the shape of the cell. It is often, however, somewhat irregular, and in long cells, such as the cells of columnar epithelium, it is elongate. The surface of the nuclei of very active cells is sometimes increased by the formation of lobes, and in the spinning glands of certain insects such lobes become highly branched and ramify throughout the cell (fig. 2). In this way a large surface is presented for interaction between the nucleus and the cytoplasm.

It has been found that a quantitative relationship exists between nuclear mass and cytoplasmic mass; this is known as the *karyoplasmic*

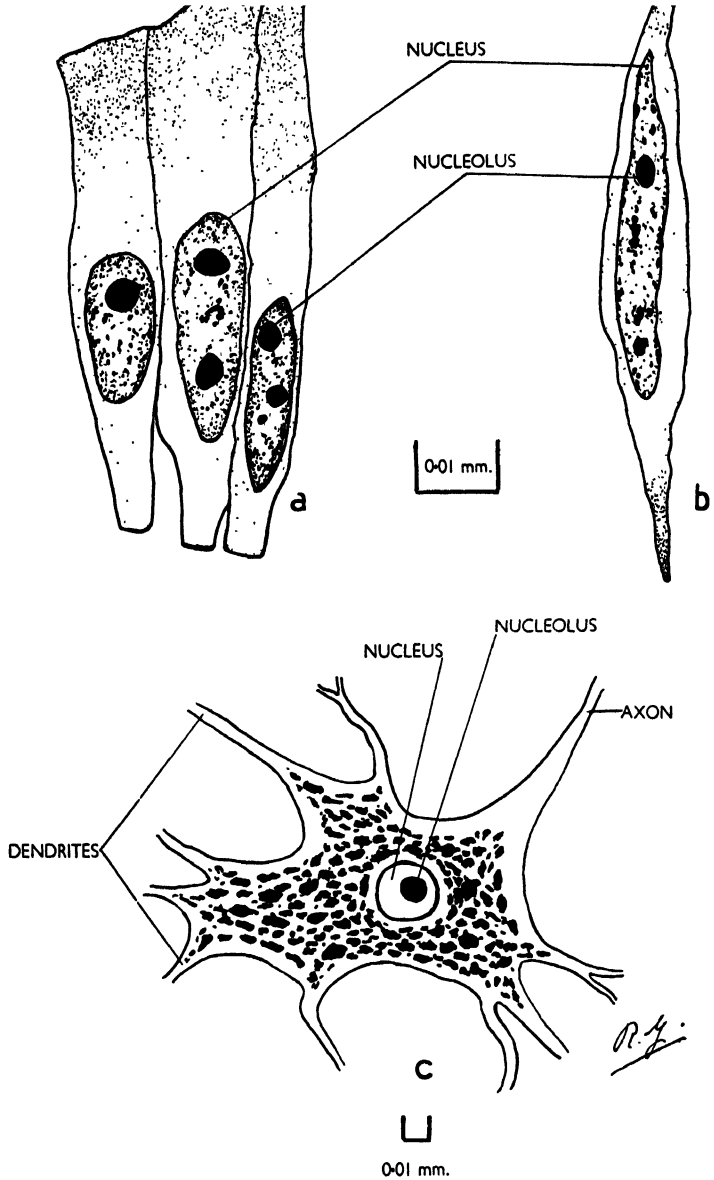


FIG. 1.—a, three columnar epithelial cells from the mucous membrane of the small intestine of the frog. b, unstriped muscle cell from the small intestine of the frog. c, multipolar nerve cell from the ventral horn of the spinal cord of the ox.

ratio. In the cleavage divisions of annelids and of molluscs inequalities of cytoplasmic division occur while nuclear division is equal. The nuclei increase in volume until they reach a size that is roughly proportional to that of the cytoplasm. It has been shown that the size of the nucleus is proportional to that of the undifferentiated active cytoplasm, and not to that of the whole cell which may contain a large amount of yolk.

The nuclei of undifferentiated cells, such as embryonic cells and the early stages of the germ-cells of animals, are generally relatively large. As differentiation proceeds, the relative volume of the nucleus decreases. The relative size of the nucleus appears also to depend on the stage of the division cycle. During the interphase the nucleus increases in size and is largest in the late prophase immediately prior to the disappearance of the nuclear membrane. It would appear, therefore, that the large size of the nucleus in certain cells, which divide infrequently, is correlated with the length of the interphase.

It has been shown experimentally that the size of the nuclei of the eggs of sea-urchins is proportional to the number of chromosomes present, and that the karyoplasmic relations may be restored by an increase or a decrease in the number of cleavages. Variation of chromosome number cannot be a factor of great importance in normal cleavage. It would appear that the main factors which determine the normal size relations between the nucleus and the cytoplasm are the length of the interphase and the amount of undifferentiated cytoplasm present in the cell.

The cells in the body of a multicellular animal or plant are, with few exceptions, derived from the fertilized ovum. The latter by successive divisions gives rise to the cells of the embryo, and these, in turn, divide repeatedly and undergo differentiation to form the tissues which make up the body of the adult organism. Some of the cells of the adult become highly specialized and are incapable of further division. Others remain relatively undifferentiated, retain some of their embryonic characters, and undergo multiplication to replace those worn out through the ordinary metabolic processes or are lost by other means. Cell division is usually followed by growth which consists of the elaboration of new protoplasm, and is brought about by the activity of the living substances of the cell. Some cells, for example the eggs of animals, increase greatly in size through the elaboration and accumulation of non-living products of cell activity. As interchange must continually take place between the nucleus and the cytoplasm, and as the area of the nuclear membrane does not increase at the same rate as the volume of the nucleus and the cytoplasm, it is probable that division occurs when a certain ratio is established between the area of the membrane and the volume of the nucleus and the cytoplasm. In other words, a stage is reached when the interchanges between the nucleus and the cytoplasm are not sufficient to allow for the further growth of the cell. It is also probable that, through the increase

FIG. 2.—Photomicrograph of a small part of the spinning gland of the larva of *Liparis*. To show the branched nuclei; the outlines of the cells are not visible. × 205.

in size of the cell, the area of the cell membrane becomes insufficient for the necessary exchanges with the external environment.

We have seen that the cells of the embryo, and new cells in the adult, become differentiated and specialized to form the various tissues of the body. During this process the cells undergo changes of form and of character which enable them to perform special structural and physiological functions. At the same time differentiation frequently occurs within the cell itself so that certain functions are localized in definite regions.

In conclusion: the cell is a structural and functional unit; the tissues of the bodies of animals and plants are composed of cells or the products of cells; the various tissues are usually derived by division from the fertilized ovum. It must, however, be clearly understood that non-cellular structures exist in the bodies of multicellular organisms, that protoplasmic connections between neighbouring cells have been described in certain cases, and that binucleate and multinucleate forms occur amongst the Protozoa and are the rule in the majority of the filamentous Fungi. While the unspecialized cell of a multicellular organism is capable of carrying out the activities characteristic of living matter it is dependent upon the external environment. An animal cell takes in oxygen from the surrounding medium, absorbs nutritive material from the blood or other fluid, or from neighbouring cells, eliminates waste matter, reacts to stimuli, exhibits conductivity, grows and reproduces. There are delicately balanced interactions between its various parts and between the cell and its surroundings. It forms part of an organism and is intimately dependent upon the life of the organism as a whole; at the same time it reacts upon the life of the organism as a whole.

A BRIEF HISTORY OF CYTOLOGY

Our knowledge of the structure of the cell has been slowly built up as the result of a long series of investigations, and in recent years improved technique has opened new fields of research, or methods of approach, which have added to the understanding of the minute cellular components and inclusions. In a work such as this it is not possible to deal fully with the history of cytology; the following short sketch will, however, be sufficient to draw attention to the main developments and to indicate some of the problems awaiting elucidation. Further notes on the development of certain aspects of cytology will be found in the relevant sections.

The term "cell", as first employed by Robert Hooke in 1665, meant a hollow chamber surrounded by definite walls; consequently the wall was considered to be the most important part of the cell. It was later realized that cells are not always empty spaces, and in the early nineteenth century the importance of the cell "juice" received some recognition. In 1759

Wolff recognized that the tissues of plants and animals are composed of " spheres " and " vesicles ", and also that the embryo is produced from the fertilized egg by the progressive production of new parts. The nucleus was first seen by Fontana in 1781 ; that it is a characteristic cell structure was recognized by Meyer in 1826, but its discovery is usually attributed to Robert Brown who, in 1833, observed a body in plant cells which he called the nucleus. These and many other investigations preceded the Cell Theory of Schleiden and Schwann. In 1838 Schleiden formulated the conception that cells are the structural units of plants, and in 1839 Schwann proposed a similar theory for the structure of animals.

The Cell Theory at first contained a number of erroneous conceptions and assumed its modern form as the result of researches carried out subsequent to its inception. Wilson pointed out that the history of cytology since the first formulation of the Cell Theory may be divided into three periods. In the first period, 1840–70, " the fundamental outlines of the cell-theory were marked out and the principals of genetic continuity became more clearly defined ". The second period, 1870–1900, " included a development of cytology and cellular embryology which gave more definite form to our general ideas concerning the physical basis of heredity and the mechanism of development ". The third period opened with the rediscovery of Mendel's work on heredity in 1900.

The development of our knowledge of cell structure and physiology was slow, and is still far from complete. The name " protoplasm " was first used by Purkinje in 1840, and, due to a marked improvement in cytological fixatives between 1870 and 1890, various theories of protoplasmic structure were propounded (p. 10). The development of technique yielded important results, but many of the interpretations of cell structure were erroneous, due to a failure to distinguish between the structure of living protoplasm and the appearance of fixed and stained preparations.

Cell division was seen by several observers prior to the formulation of the Cell Theory. Its occurrence was recognized by Schleiden and Schwann, but they believed that new cells are usually formed by a process of " free cell-formation " from a continuous matrix—the " cytoblastema ". In 1844 Kölliker claimed that new cells arise only by division of pre-existing cells. He stated later, however, that " free cell-formation " also occurs. That cell division is the only method of origin of new cells was again stated by Remak in 1852 and by Virchow in 1855, and the genetic continuity of cells was finally established.

The discoveries that the egg and the sperm are single cells, that the sperm enters the egg, and that the nucleus of the sperm fuses with the nucleus of the egg, marked important stages in the history of cytology. Sperms had been seen by many of the early observers, but their function in fertilization was not understood. In 1841 Kölliker showed that sperms

were formed by the metamorphosis of cells within the testis, Gegenbaur in 1861 stated that the egg is a single cell, and in 1865 Schweigger-Seidel and La Valette St. George demonstrated that sperms contain cytoplasm as well as a nucleus. The entry of the sperm into the frog's egg was first described by Newport in 1854, and in 1875 Hertwig showed that the sperm-nucleus fuses with that of the egg.

Owing to the improvements in methods of fixation referred to above, comprehensive studies were carried out on cell division and the behaviour of the chromosomes during mitosis and meiosis. In 1883–87 Van Beneden followed the history of the nuclei in the fertilized ovum of *Ascaris* and showed that the chromosomes of the embryo are derived in equal numbers from the male and female parents. This discovery presented new fields of research, particularly in relation to the cytological study of heredity. Its importance was immediately recognized by Weismann, of whom Wilson said—" to him, therefore, above all others, belongs the credit for having placed the keystone between the study of cytology and that of heredity, thus finally bringing the cell-theory and the evolution-theory into organic connection "

These and other investigations on cell division, cellular embryology, and the structure and physiology of the cell, formed the basis for the remarkable advances of the next century.

In 1900 Mendel's work on heredity was rediscovered, and as the result of earlier cytological research it was soon recognized that the behaviour of the chromosomes gave an explanation of the method of distribution of the hereditary units, or genes, amongst the gametes, and the combination of maternal and paternal genes in the nucleus of the fertilized egg. Important contributions to the subject were contained in the papers of Boveri, Montgomery and others; Sutton in 1902 and 1903, and De Vries in 1903, gave a complete explanation of the behaviour of the chromosomes in relation to the work of Mendel. In 1901 and 1902 McClung suggested that the chromosomes are concerned with the determination of sex, and this was confirmed in 1905 by the work of Stevens and of Wilson. Research on the chromosomes in relation to reproduction and heredity rapidly developed and led to the modern science of *cytogenetics*—so productive of results, of which the most important, in the light of recent investigations, will be outlined in later sections of this work.

Meanwhile notable advances were made in our understanding of the components of the cytoplasm. The discovery of the Golgi material in 1898, and the investigations of Altmann, Benda, Meves and others on the mitochondria led to much valuable work. Improvements in the methods of demonstrating the cell components in fixed material and, to a limited extent, the use of vital dyes have opened a vast field of research, particularly in the study of germ-cells and gland-cells. The contributions

to the subject are too numerous to be mentioned here, but it should be noted that both mitochondria and Golgi material are present in practically every type of animal cell examined with suitable methods of technique, and that there is evidence that they play an important part in cellular activities The main contributions to this branch of cytology will be dealt with later.

In recent years there has been a marked improvement in the methods of examining the various cell structures. Not only has there been a certain advance in methods of fixation and staining, and a recognition of the dangers inherent in basing conclusions on one method of fixation alone, and of the desirability of examining living material whenever possible, but some entirely new methods of approach have been evolved.

By means of the microdissection apparatus, or micromanipulator, it is possible to dissect a living cell contained in a drop of sea water, physiological saline or other suitable medium, and to obtain valuable information regarding the physical characters of the protoplasm and of its various components. The development of methods of cultivating tissues outside the body has yielded information regarding cell division, and work has also been carried out on the cytoplasmic components as seen in tissue culture cells. Among other methods used with success, the histochemical analysis of cells, the use of polarized light, the electron microscope, the phase-contrast microscope, micro-incineration methods for the identification of the inorganic constituents of cells, the use of the ultra-centrifuge, and, in the experimental field, the use of X-rays, ultra-violet light and the administration of certain chemicals must be mentioned.

With the growth of our knowledge of the chromosomes and the linking of this aspect of cytology with genetics, there has been a tendency for cytologists to become divided into two schools—that of nuclear cytology in relation to genetics, and that which concerns itself mainly with the cytology of the cytoplasm. The recognition of the importance of the chromosomes as the bearers of hereditary characters, and the development of techniques which made possible extensive research in other fields, inevitably led to specialization, but it is to be regretted that the two fields, especially that of cytogenetics, tended to work for the most part in isolation. The contribution of the cytoplasm of the germ-cells to the embryo and the part which it may play in development has been for too long almost entirely overlooked. There are, however, indications of a revival of interest in problems connected with cytoplasmic inheritance. In the opinion of the writer a closer union of the two branches of cytology would yield important results, contribute to the knowledge of reproduction and stimulate fresh fields of research.

Some work has been carried out on abnormal and diseased cells, but medical science is slow to recognize the contributions which cytology can make to pathology and medicine. The animal cell is a delicately balanced

structure, in intimate connection with its immediate environment and in physiological unity with the rest of the body of which it forms a part; it is probable, therefore, that its further study in diseased tissues would throw light on some of the fundamental problems of medicine.

During recent years great advances have been made in cytology and in methods of cytological technique. As a result of these developments new problems await solution. Cytology is entering upon a new period when, through the collation of the results of various cytological investigations, a clearer understanding will be obtained of the various cell components and of the part which they play in the life of the cell. A period when, to an ever-increasing extent, cytologists will make use of the knowledge which has accumulated in other fields of science, and when cytology will help to solve some of the problems of medical science and of biology as a whole

CHAPTER II

THE PROTOPLASM

Protoplasm is a colourless heterogeneous substance. The protoplasm of the nucleus is called the *nucleoplasm* and that of the rest of the cell the *cytoplasm*. The clear fluid protoplasm of the cell is often referred to as the *hyaloplasm*; granules, globules and the various components of the nucleus and of the cytoplasm are distributed in the hyaloplasm.

Due to its complexity, the study of living protoplasm presents many difficulties, and examination by routine methods which bring about its coagulation yields results of little value. It is now recognized that only by the study of living cells and by refined methods of technique can protoplasmic structure be understood. Much information has been obtained in recent years, by the use of the microdissection apparatus, ultra-violet light, polarized light and dark-ground illumination, regarding the living components of the cell. Experiments with the ultra-centrifuge have proved of value in determining the relative specific gravities of the cell components.

There have been many theories of protoplasmic structure. The older workers believed that it has a fibrillar structure. Certain investigators claimed that the fibres are continuous and form a reticulum throughout the cell, while others believed that the fibres are discontinuous. The fibrillar theory was followed by the alveolar theory which claimed that protoplasm has an alveolar structure. According to the granule theory the protoplasmic granules were regarded as organic units which build up the cell.

There is evidence that the cytoplasm contains numerous submicroscopic particles, or *microsomes*, composed essentially of *ribose nucleoproteins* and *phospholipides* (Lazarow, 1943, and Claude, 1943). These submicroscopic particles were first isolated centrifugally by Claude, who suggested that they form an important part of the ground substance.

Certain modern workers believe that protoplasm possesses a submicroscopic fibrous structure and that the fibres consist of long protein molecules. There appears to be considerable evidence in favour of such a view; further work, however, is desirable in this important field of research.

The microscopically visible components of the protoplasm are dealt with elsewhere in this book

THE PHYSICAL CHARACTERS OF PROTOPLASM

The viscosity of protoplasm varies considerably in different types of cells and in the same cell at different stages of physiological activity. In certain cases the viscosity may be only two or three times that of water, while in others it is very much higher. Experiments with the micro-dissection apparatus have shown that protoplasm possesses considerable elasticity. Cells and parts of cells stretched by means of the micro-dissection needle, upon release regain to a considerable extent their original shape. In a similar manner it has been demonstrated that the cell membrane is elastic.

THE CHEMICAL COMPOSITION OF PROTOPLASM

Protoplasm consists of proteins, carbohydrates, fats, 75-85 per cent of water and about 1 per cent of inorganic salts. The relative proportions of these substances vary in different organisms and also in the different physiological states of the same cell.

Water plays a very important part in the life of the cell. It acts as a solvent and, through hydrolysis and dehydration, takes part in various reactions. It is of importance in the exchange of substances between the cell and its environment—for example, the exchanges between the cell and the lymph

Proteins are complex compounds containing carbon, hydrogen, oxygen, nitrogen, frequently traces of sulphur and phosphorus, and sometimes of magnesium and iron. They are built up of organic acids containing an *amino group*—NH_2, and consequently known as *amino acids*. There are many kinds of proteins depending upon the grouping of the amino acids, and different organisms differ somewhat in the nature of their proteins. During the process of digestion, in a mammal for example, the proteins contained in the food are broken down into amino acids. These pass into the blood stream, are assimilated by the cells which require them, and are built up by the action of intracellular enzymes into the proteins of the animal's body.

Special proteins (built up of proteins and nucleic acid) are present in the nucleus and the cytoplasm. They are the chief constituent of the chromosomes and are known as *nucleoproteins* (pp. 34-35). Different organisms differ in respect of the character of their nucleoproteins.

The *Carbohydrates* are compounds of carbon, hydrogen and oxygen. The starches and sugars are the best-known examples, and glycogen is common in the cells of animals. The pentoses enter into the composition of protoplasm; other carbohydrates act as sources of energy and supply building materials. By their oxidation carbohydrates are the ultimate source of energy.

Fats are composed of glycerol and fatty acids and are often present as globules and droplets. Fats form important food reserves and are readily oxidized with the production of heat. *Lipides* contain nitrogen, or phosphorus and nitrogen, in addition to the carbon, hydrogen and oxygen present in fats. Some of them, such as the phospholipides, appear to enter into the composition of the protoplasm. The phospholipides also enter into the formation of surface membranes.

The *Inorganic Salts* are held in solution in the water of the cell. They are chiefly calcium and sodium and include those present in sea water. Sodium increases, while calcium decreases, the permeability of cell membranes; hence the ratio of the concentration of the various salts is of importance.

PROTOPLASM AS A COLLOID SYSTEM

The name *colloid system* is applied to a system containing fine particles suspended in a medium; the particles will not diffuse through an animal membrane and will give formless masses of material when evaporated. Such a system differs from a crystalloid solution in which the dissolved substances will diffuse through animal membranes and on evaporation will give crystals or formed masses of material. A colloid system possesses numerous very small particles dispersed in a continuous phase. Consequently there is a very large surface between the particles of the dispersed phase and the continuous phase. As many reactions take place at such surfaces, the colloidal nature of protoplasm is of primary importance. A colloidal system is said to be in the *sol* condition when it is fluid and flows readily; when more solid in nature it is known as a *gel*.

Protoplasm is a complex colloidal system which usually behaves as a viscous liquid. While in most cases protoplasm may be regarded as a colloidal sol, under the influence of internal and of external stimuli it may change from the sol to the gel condition. This action is reversible, and takes place without any permanent interruption of the vital activities. It is well illustrated in amoeboid movement. In *Amoeba* the ectoplasm and the outer part of the endoplasm is in the gel condition, and the inner part of the endoplasm is in the sol state. There is a change from plasmagel to plasmasol at the point where movement is about to take place. The sol so formed flows backwards and returns to the gel state. At the same time, the plasmagel at the region farthest away from the direction of movement is converted into plasmasol and flows forward under the pressure of the plasmagel. In this way movement is brought about and there are reversible changes between the gel and the sol conditions.

It will be seen that protoplasm is an extremely complex substance. It is made up of many substances combined in the cell, and organized so that the cell acts as a whole. Due to the physical and chemical properties

of the protoplasm, delicate interactions take place between the various parts of the cell and between the cell and its external environment. The minute structure of the protoplasm is, however, not yet fully understood. The chemical and physical properties of some of the components of the nucleus and of the cytoplasm are discussed in the subsequent chapters.

CHAPTER III

THE STRUCTURE OF THE ANIMAL CELL

THE *nucleoplasm* and the *cytoplasm* contain structures which are part of the living material of the cell; inclusions which are formed as the result of the activities of the protoplasm may also occur. The present chapter is a generalized account of the animal cell (fig. 3). In the sections devoted to special aspects of cytology some of the cell components will be described in greater detail.

THE RESTING NUCLEUS

The term "*resting nucleus*" is usually applied to the non-dividing nucleus. It must be understood that the "resting nucleus" is not one in which the metabolic activities are reduced, but that it is only resting in the sense that it is not in the process of division. The nucleus is at all times extremely active physiologically, and there is evidence that it exerts a controlling influence on the cell as a whole, and that chemical exchanges take place between the nucleus and the cytoplasm.

When a cell is subjected to the action of a fixing agent, some of the cell constituents are coagulated, and the picture of the nucleus so produced is often very different to that presented by preparations of living cells. Therefore, in order to study the structure of the nucleus, or of the cell as a whole, it is necessary, whenever possible, to examine both fixed and living material. In fixed and stained sections the following nuclear structures are visible—the *nuclear sap*, the *nuclear network*, *chromatin granules*, *nucleoli*, and in certain cases parts of *chromosomes*.

The NUCLEAR NETWORK is described as a net-like reticulum of lightly stained material. Granules or masses of deeply stained material, or *chromatin*, are scattered on the network. In preparations of living cells the network and granules are not visible, and it is now recognized that they are artifacts produced by the action of chemicals in the fixing fluid on certain of the nuclear constituents. In the living nucleus the only structures seen clearly are—the nuclear sap, nucleoli and in some few cases thin thread-like chromosomes, or parts of chromosomes.

The NUCLEAR SAP is usually a clear fluid with a fairly low viscosity In some cases it may be in the gel condition.

THE CHROMOSOMES.—Generally speaking, the chromosome number

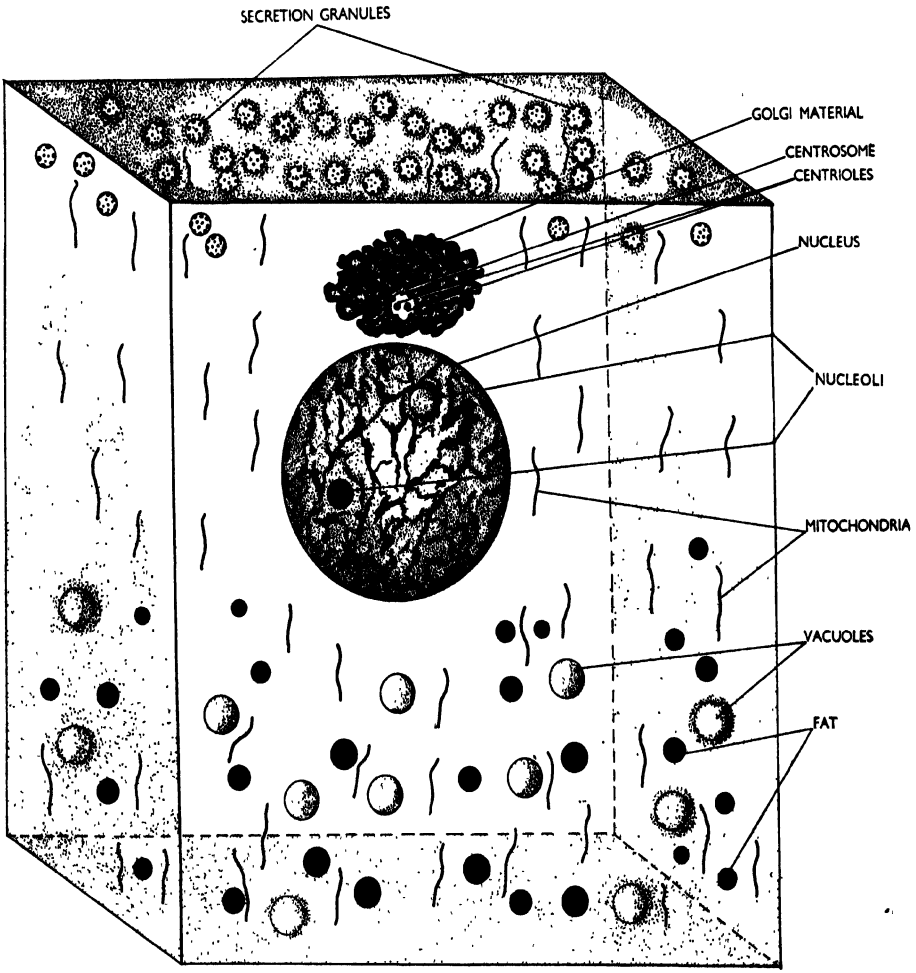


FIG. 3.—Generalized diagram of an animal cell. The Golgi material is shown in the localized condition surrounding the centrosome. The mitochondria are represented as scattered through the cytoplasm.

is constant in all the individuals of a species (p. 34). It was formerly believed that the chromosomes disappear at the end of each nuclear division and re-form at the beginning of the next mitosis. There is considerable evidence that the chromosomes maintain their individuality from division to division—that the same chromosomes which become invisible at the end of one division reappear at the beginning of the next. Briefly, these conclusions are based on—(a) an optically identical set of chromosomes are handed on from cell to cell; (b) genetical evidence regarding the distribution of the hereditary factors, or genes; (c) in a few cases the chromosomes are said to reappear in the position which they occupied at the end of the preceding division; for example, during the cleavage stages of *Ascaris* the ends of the telophase chromosomes lie in lobelike projections of the nucleus. The lobes persist throughout the " resting stage ", and at the prophase the ends of the chromosomes become visible in the lobes which they occupied at the end of the preceding telophase. It is now generally accepted that the chromosomes persist throughout the " resting stage ", and this is supported by the isolation of chromatin threads from the non-dividing nuclei of leukemic cells (Claude and Porter, 1943).

THE NUCLEOLI.—One or more may be present. They vary in staining properties in nuclei of different types of cells and in the same nucleus at different phases in the life of the cell.

THE NUCLEAR MEMBRANE.—The nuclear membrane is a thin limiting membrane. Experiments with the microdissection needle have proved that the membrane is a definite structure. It offers resistance to the needle and can be indented under pressure; it will regain its original shape when the pressure is released.

THE CYTOPLASMIC STRUCTURES

The examination of the cytoplasmic structures of living cells presents many difficulties. The existence of the mitochondria has been confirmed by observations with vital dyes, and the Golgi material is visible in certain types of living cells.

The PLASMA MEMBRANE, like the nuclear membrane, possesses definite physical properties and plays an important part in the regulation of the exchanges which take place between the cell and its environment. Its existence has been proved by means of the microdissection apparatus; if slightly damaged with the microdissection needle it is renewed from the cytoplasm.

THE CELL SAP.—Optically, the *cell sap* is usually a homogeneous substance; chemically it is extremely complex. In the eggs of echinoderms it appears to consist of a homogeneous phase in which are situated large spheres and small granules. The coarse network often seen after

treatment with certain fixatives is an artifact. The various cytoplasmic components and products are distributed in the sap.

THE DIVISION CENTRES.—The *division centre*, or *centrosome*, is usually in the form of a rounded body which, in the "resting stage", lies at one pole of the nucleus. It contains a small and deeply stained granule—the *centriole*. In many cases the centriole is a permanent component of the cytoplasm, but in some instances it appears to arise *de novo*. As the centriole often divides shortly after nuclear division the centrosome of the "resting cell" may contain two granules; other variations from the usual type are known. Prior to cell division the centrosome divides and each half eventually takes up a position at opposite poles of the nucleus. The division of the centrosome is preceded by the division of the centriole, and this may take place just before nuclear division or shortly after the preceding mitosis. In the "resting cell" the centrosome is surrounded by an area of modified cytoplasm known as the *idiosome* or *archoplasm*. This substance is not clearly visible with routine methods of technique.

THE SPINDLE.—In fixed preparations of dividing cells fibres are seen forming a spindle-shaped body lying between the centrosomes. It is generally believed that spindle fibres are not visible in normal living cells. They are produced by adding acid to the medium, and on removing the acid the fibres disappear; as most fixatives contain acid, it is stated that the fibres are artifacts. There has been considerable controversy as to the reality of the fibres, and various theories have been put forward to explain their presence in fixed material. Schrader (1944) believes in the reality of spindle fibres and states that they have been seen in the living cleavage cells of a mite and in certain flagellates. Hughes-Schrader and Ris (1941) claim to have seen fibres in living material of the coccid *Steatococcus*, but Schrader observes that "until such cells are shown to complete a regular mitotic cycle (as these did not) the evidence is affected by the possibility that the conditions are not entirely normal". Schrader cites Schmidt's work with polarized light, and states that this worker has produced convincing evidence of longitudinal differentiation in metaphase spindles.

In fixed material the spindle appears to be made up of *continuous fibres* stretching from pole to pole, and of *half spindle fibres* corresponding in number to that of the chromosomes and extending from the spindle-poles to the chromosomes (fig. 4). It is claimed that short fibres, or *interzonal connections*, stretch between the anaphase chromosomes of some animals The interzonal connections vary in structure and are possibly derived from the chromosomes (Schrader, 1944). Schrader claims that in some cases the half-spindle fibres arise as the result of an interaction between the spindle poles and the centromeres (p. 31) while in others they are formed chiefly or wholly through the activity of the centromere. He believes that

there are two main types of spindles; the direct type in which the chromosomes are connected with the poles by chromosomal fibres (half-spindle fibres), and the indirect type in which the chromosomes are connected with a continuous fibre, possibly through a chromosomal fibre.

In living material the spindle generally appears as a homogeneous hyaline structure. It can be moved through the cytoplasm by centrifugal force and by means of the microdissection needle. It is believed that the spindle is usually formed in two parts. A part arises between the centrosomes before the disappearance of the nuclear membrane; it is cytoplasmic in origin and is called the *central spindle*. After the nuclear membrane disappears the central spindle moves into the nuclear region. Nuclear material undergoes gelation around the central spindle to form the *half-spindle fibres*, or *attachment elements*. In the metaphase the chromosomes, attached to the half-spindle fibres, are situated around the periphery of the equator. Bernal (Schrader, 1944) suggests that the spindle may consist of protein molecules arranged in parallel lines, and that the spindle fibres are formed from small quantities of the outside medium which are included in the spindle. Later, the whole system elongates and brings about the movement of the chromosomes.

In some animals, such as *Artemia* (Gross, 1935), certain Protozoa and scale insects, the whole spindle is nuclear in origin. In these cases some of the chromosomes may lie in the middle of the spindle.

THE ASTERS.—During the early changes which precede nuclear division rays develop around the centrosomes (fig. 4). These are known as the *astral rays*, and, together with the spindle, form the *amphiaster*. The aster can be moved through the cytoplasm by means of the microdissection needle and the individual rays can be bent and twisted. The rays are broadest at their base and consist of a fluid of relatively low viscosity. The whole aster possesses a certain rigidity due to the presence of granular cytoplasm between the rays. It is probable that the rays consist of protoplasmic streams which move in an outward direction, and it has been suggested that the long protein molecules of the protoplasm become arranged in rows parallel to the lines of protoplasmic flow (Pollister, 1941).

THE MITOCHONDRIA.—The *mitochondria*, or *chondriosomes*, are in the form of rods, granules, threads and sometimes of spheres (figs. 3 and 5). The granules are often referred to as *chondriomites*, the rods and threads as *chondrioconts*, and the spheres as *chondriospheres*. In living-tissue culture cells mitochondria have been observed undergoing changes of form—rods breaking up into granules, and granules becoming aligned to form threads which may shorten and form rods. With the aid of dark-ground illumination mitochondria of tissue culture cells have been seen in constant movement which is of two types: they undergo a wriggling movement and, at the same time, move from one part of the

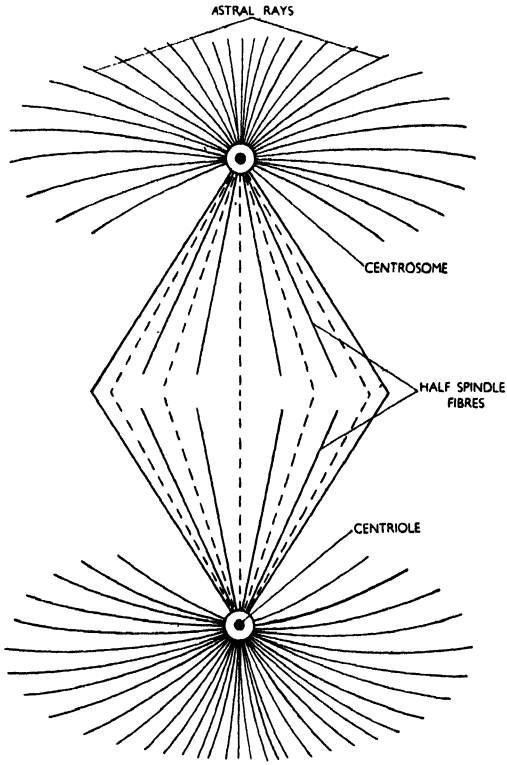


FIG. 4.—Diagram illustrating the structure of the spindle in an animal cell.

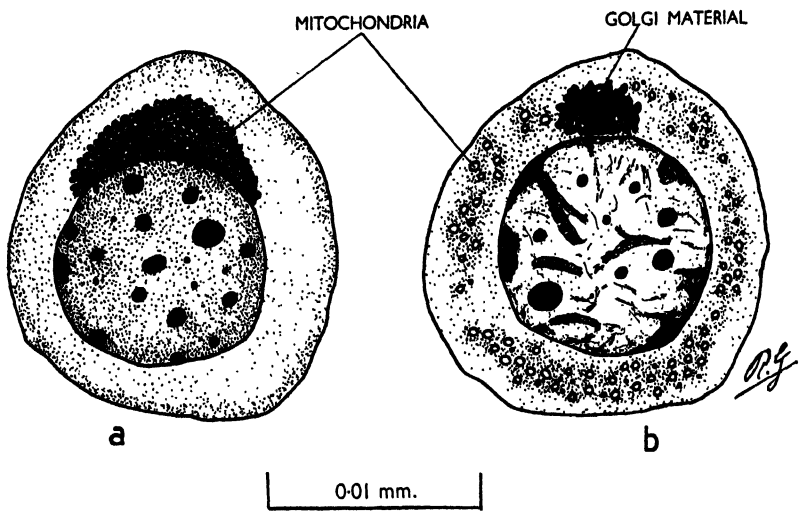


FIG. 5.—Primary spermatocytes of the pig. Original drawings. a, the spherical mitochondria are shown surrounding the Golgi material. b, an older spermatocyte ; the mitochondria are distributed through the cell ; the Golgi material surrounds the centrosome.

cell to another. It is possible to stain the mitochondria of the living cell with vital dyes such as Janus Green B.

The mitochondria are usually scattered through the cell (figs. 3 and 5, b) but in certain phases of cellular activity they may be collected into clumps (fig. 5, a) or they may be particularly numerous in certain regions of the cytoplasm. There is reason to believe that they play an important part in the activities of the cell and probably in the elaboration of secretion granules. Chemically the mitochondria are thought to consist of a lipoid cortex which surrounds a core of protein, but it is probable that proteins as well as lipoids are present in the outer part. Mitochondria are destroyed, or imperfectly preserved, with routine fixatives which contain acetic acid or other fat solvents. Special fixatives are used for their preservation.

THE GOLGI MATERIAL.—The *Golgi material* was first described by Golgi in 1898 in the spinal ganglion cells of vertebrates which had been treated with silver nitrate. It had, however, previously been observed by Platner in 1885 and by Hermann in 1891. It was first described as a net-like structure composed of fibrils, and was called the *internal reticular apparatus of Golgi*. Later, it received the name of *Golgi apparatus*. It is now recognized that this material is of widespread occurrence and it has been demonstrated in practically every type of animal cell. It frequently occurs in the localized condition, lying beside the nucleus and having the appearance of a network (figs. 3 and 5, b). On close examination the net-like structure often appears to be made up of individual elements which do not join to form a network, but are closely clumped together around the archoplasm. Golgi material is frequently scattered through the cell. In some cases the elements of the localized Golgi material spread out through the cytoplasm, but may come together again at a later stage. Such movements are correlated with the activity of the cell. The scattered elements of the diffuse condition are called *Golgi bodies*, *Golgi elements* or *dictyosomes*. As there is some doubt as to the existence of a true network, some cytologists use the term *Golgi material*, or *Golgi substance*, rather than Golgi apparatus. These terms are preferred by the writer, although it is recognized that a true network may exist in neurones and in certain other cells.

The Golgi material is believed to play an important part in the formation of secretion granules and of other materials. It appears to consist of two parts, an osmiophilic and argentophilic cortex, and an osmiophobic and argentophobic inner region. The Golgi material is probably composed of protein and lipoid substances, and is not preserved in material treated by the ordinary methods. In ultra-centrifuged cells it forms a layer distinct from the mitochondria. Special osmium tetroxide and silver nitrate techniques are used for its preservation, and it has been seen in living unstained cells of certain tissues.

THE METAPLASM.—The cytoplasm contains a number of inclusions which are not part of the living protoplasm, but are formed as the result of the activities of the living substance. The *metaplasm* (fig. 3) consists of such materials as secretion granules, yolk globules, fat droplets, glycogen, protein crystals, pigment granules, etc., often present in the animal cell.

CHAPTER IV

THE STRUCTURE OF THE PLANT CELL

A GENERAL account has already been given (Chapter III) of the animal cell, and, as much that has been written there applies equally to the plant cell, repetition is unnecessary. Each consists of nucleoplasm and cytoplasm enclosed by the semi-permeable, elastic plasma membrane, and

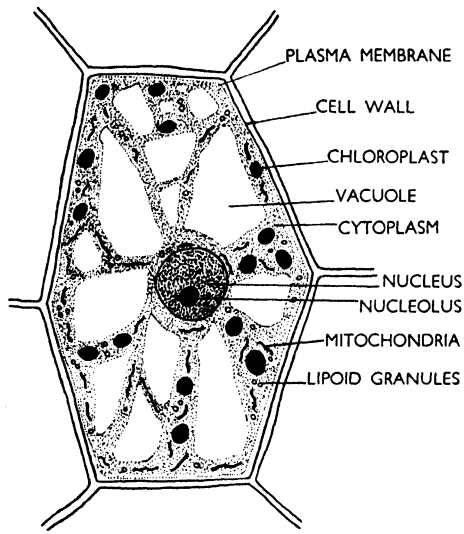


FIG. 6.—Generalized diagram of a typical plant cell.

each contains elements considered to be part of the living material of the cell and inclusions which are the products of the metabolic activities of the protoplasm (fig. 6). There are, however, certain features which are either peculiar to plant cells or are of particular interest, and these will be discussed here.

THE CELL WALL

Although naked protoplasmic structures do occur, *e.g.* the zoospores and gametes of certain Algae, the plasmodia of the Slime Fungi (Myxomycetes) and cells arising during the reproductive processes in vascular

plants, the protoplast of the plant cell is normally enclosed by a conspicuous cell wall. This wall, by virtue of its apparent direct origin from the cytoplasmic cell plate at the close of mitosis, was at one time thought to be part of the living material of the cell. Later, it was believed that the cell plate split to form the plasma membranes of the daughter cells and that the primary wall or *middle lamella* was laid down between them. There is still no general agreement concerning the exact method of wall formation, but more recent investigation has shown that the cell plate is a fluid film and it seems likely that the deposition of calcium pectate within this film converts it into the primary wall. Protoplasmic connections, the *plasmodesma*, most frequent, apparently, in those tissues where metabolic activity is at its highest, penetrate intervening walls and bring into contact the protoplasts of adjacent cells. Although not always easy to demonstrate, it is thought that such protoplasmic continuity is a constant feature of living plant cells.

As differentiation of permanent tissues proceeds, the young cells enlarge and their walls increase in extent. This increase is brought about, partly by the natural extensibility of the material of the primary wall and partly by the deposition of new cell wall substance either upon (*apposition*) or within (*intussusception*) the framework of the original wall. This process continues until the cell has more or less reached full size, when a secondary layer, usually differing chemically from the primary, is laid down. It differs further in that it is not continuous and even in its most developed state is always interrupted by thin areas or *pits* where the only wall is the primary one. In conducting elements, *e.g.* tracheids and vessels, these pits become partially overhung on each side by circular ledges while the *closing membrane* (*i.e.* the primary wall) becomes thickened and chemically altered to form the *torus*. The complete structure is known as a *bordered pit* in contrast to the *simple pit*. Changes of pressure in adjacent cells alter the position of the torus so that the aperture of the pit may be closed and the passage of water from one vessel to another prevented.

The chemical basis of all cell walls is the carbohydrate, *cellulose*. Pectic substances, usually soluble pectin (pectinogen) and less commonly insoluble pectin (pectose) which may be present in combination with metallic salts of calcium, magnesium or iron, are found in all thin-walled tissue.

As cells mature, various chemical changes may take place in the composition of their walls. On the outer surface of the epidermal cells of the aerial parts of most vascular plants, a continuous layer of *cutin*, broken only by the stomatal pores, is deposited. This, like the *suberin* of cork cells, is a mixture of many complex compounds, including condensation and oxidation products of certain unsaturated fatty acids and the derivatives of these products. The presence of a thick *cuticle* may

greatly reduce water loss through exposed cell walls, while suberised walls are completely impervious to water.

Cells in which the original cellulose wall has been modified by the deposition of *lignin* occur in the conducting and strengthening tissues of vascular plants. Such lignified walls are harder and stronger than those of unmodified cellulose, have less elasticity and are less readily permeable to water. They are impermeable to air. The composition of the large molecule of lignin has not yet been elucidated, but it seems probable that the phenolic alcohols, coniferyl alcohol and cinnamic alcohol are the basis of its structure. With the lignin in lignified walls are often found fats, resins, gums and tannins.

Cells with mucilaginous walls, which are hard and horny when dry but which absorb large quantities of water when wet are found in water and food storage tissues, in the coats of seeds and fruits and in seaweeds.

Various mineral substances may occur in cell walls. The walls of the epidermal cells of Grasses, Horsetails and Sedges, for example, contain silica; the cell walls of Diatoms are composed entirely of pectin and silica, while clusters of crystals of calcium carbonate (*cystoliths*) suspended on special outgrowths of the cell wall are characteristic of certain families of Flowering Plants, *e.g.* the Urticaceae and Moraceae.

THE CYTOPLASMIC STRUCTURES

THE CENTROSOME.—The centrosome is not a characteristic feature of the typical plant cell, although it occurs in most of the motile Algae, in a small number of the non-motile species (*e.g.* certain Chlorophyceae, Phaeophyceae and Rhodophyceae), in the Fungi and during the divisions leading up to the differentiation of the antherozoids in the Bryophyta, Pteridophyta and certain Spermaphyta (Cycadales and Ginkgoales).

The centrosome of the Algae, which in the higher forms is only recognizable at the time of nuclear division, always divides at mitosis and occupies the poles of the spindle. Its presence in the cells of the sedentary species is considered by Fritsch (1935) as a possible indication of their motile ancestry for, in the motile forms, centrosomes are always concerned with the development of the flagellar apparatus.

The achromatic figure of the Fungi is typically amphiastral. Of special interest is the rôle of the centrosome in the formation of the ascospores in the Ascomycetes. Following the third division within the ascus, each of the eight nuclei develops a beak at the apex of which the centrosome lies. From this, distinct astral rays curve around the nucleus, and along the line of these rays the spore membrane is deposited. At the end remote from the centrosome, vacuoles in the cytoplasm may take some part in the delimitation of the spore.

In Chapters X and XI the behaviour of the centrosome (blepharoplast)

during spermatogenesis in the Bryophyta, Pteridophyta and those members of the Spermaphyta with motile male gametes, is described (fig. 54, c). Here, again, the blepharoplast is definitely associated with the development of the organs of propulsion.

THE PLASTIDS.—Intimately concerned with carbohydrate metabolism, the plastids are found in the cells of all groups of plants with the exception of the Bacteria, the Blue-green Algae (Cyanophyceae), the Slime Fungi (Myxomycetes) and the Fungi.

The types usually recognized are the colourless *leucoplasts*, the chlorophyll containing *chloroplasts* of the green parts of plants and the pigmented *chromoplasts* of flowers and fruits. All appear to originate from small granular or rod-like *plastid*-primordia or *proplastids*, found in the embryo and in the cells of meristematic tissue. They multiply by division and are distributed between the daughter cells at mitosis. There is still doubt as to whether the plastid primordia represent permanent inclusions of the cytoplasm, which can only arise by division of pre-existing primordia, or whether they are capable of being formed *de novo*.

Colourless, undifferentiated plastids (leucoplasts), such as are found in meristematic tissue, may become the more specialized *amyloplasts* and assist in the condensation of hexose sugars to starch. In the aerial parts of plants, under the influence of light and iron, leucoplasts may build up chlorophyll and, as chloroplasts, play an important rôle in photosynthesis. Chromoplasts, in which the carotinoid pigments, xanthophyll and carotin occur, are found in the flowers and fruits of many plants (fig. 7). The light-sensitive reddish *eye-spot* or *stigma* present in the motile cells of various Algae is believed by some workers to be a form of chromoplast. As, however, it does not normally multiply by division but arises *de novo* in daughter cells, this view seems to be based on insufficient evidence. *Elaioplasts*, which elaborate oil, are reported in a number of Angiosperms, but little is known concerning them.

The chloroplasts of the Algae (*chromatophores*) occur in all parts of the thallus and are often, as in the cases of the spiral chloroplasts of *Spirogyra* and the stellate of *Zygnema*, highly differentiated. They frequently enclose colourless refractive bodies, the *pyrenoids*, which seem to be centres of starch formation. The history of the algal chloroplasts has been followed from the fertilized oosphere and it is known that they are transmitted from cell to cell by division. Their behaviour, however, during fertilization is still obscure although, in some cases at least, *e.g.* *Spirogyra* and *Zygnema*, the chloroplasts of the male gamete degenerate immediately after gametic fusion.

Four pigments are normally present in the chloroplasts of the higher plants, the green chlorophylls *a* and *b*, the orange carotin and the yellow xanthophyll. It has been suggested by Willstätter and Stoll that these pigments exist as hydrosol colloids dispersed in the protoplasmic basis of

the chloroplast. Hubert (1935), on the other hand, supports the more recent view that chlorophylls *a* and *b* are chemically combined with protein to form a complex chromoprotein which, together with lipoids and the carotinoid pigments, is deposited within the colourless cytoplasmic stroma of the plastid. Zirkle visualizes the chloroplasts as vacuolated porous structures enclosed in clear colourless cytoplasm in which the pigments are uniformly distributed. For a further account of plastids and their pigments see Thomas (1940).

MITOCHONDRIA.—The presence of mitochondria in the cells of plants was first noted by Meves in 1904, and since then they have been demonstrated in all plant groups except the Bacteria and the Blue-green Algae (Cyanophyceae). Chondriomites, chondrioconts and chondriospheres (Chapter III) all occur, and, as in animals, one form may be converted into another. In young cells the granular forms are the most common, whereas chondrioconts are characteristic of cells which have undergone differentiation.

In living plant material the mitochondria seem to be slightly more refractive than the surrounding cytoplasm. They are slowly moved by cytoplasmic currents and can be seen to undergo changes of shape.

The mitochondrial complex (*chondriome*) of the Fungi has been extensively investigated (Guilliermond, 1941). In most groups, elongated chondrioconts which lie in the cytoplasm parallel to the longitudinal axes of the hyphae, are encountered, but in the Myxomycetes and Plasmodiophoraceae only granules and short rods occur. In certain genera (*e.g.* Saprolegnia, Achlya and Leptomitus) the development of the chondriome in living plants, from the germination of the zoospores to the production of zoosporangia has been followed. As the result of this investigation, Guilliermond is convinced that mitochondria are permanent inclusions of fungal cytoplasm, which are transmitted by division from cell to cell. Some investigators, however, still believe that mitochondria can arise *de novo*.

The relationship between mitochondria and plastids in the green plant has long been a problem of particular interest. In 1910, Pensa formulated the theory that chloroplasts were derived from mitochondria on which chlorophyll accumulated, and in 1911 Lewitsky concluded that plastids arise from mitochondria which are themselves of cytoplasmic origin. Guilliermond is of the opinion that two groups of mitochondria exist in the plant cell (fig. 8). Those of one group, the *inactive* or *genuine chondriosomes*, correspond exactly to the mitochondria of animals and fungi, while those of the other, peculiar to chlorophyll-containing plants, are the true pro-plastids. He believes that both types have the same characteristic forms, the same viscosity and refractivity, and both react in the same way to chemical reagents and dyes. It is therefore impossible to tell them apart when they occur together in meristematic tissues. The

FIG. 7.—Chromoplasts in the mesophyll of the fruit of *Rosa canina*. After Guilliermond, redrawn.

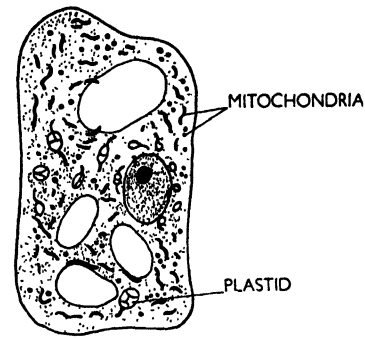


FIG. 8.—Cell from the central cylinder of the root of *Ricinus communis* showing mitochondria and plastids forming starch. After Guilliermond, redrawn.

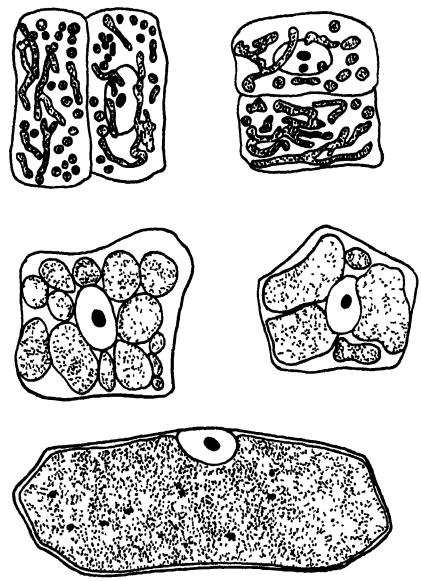


FIG. 9.—Stages in the development of the vacuolar system in the Barley root. After Guilliermond, redrawn.

FIG. 10.—The so-called Golgi material in the meristem cells of the root of *Vicia faba*. After Scott, redrawn.

pro-plastids differ from genuine mitochondria in that they are centres of active elaboration or accumulation of starch, chlorophyll and the carotinoid pigments. This modification is only transitory however, for amyloplasts may lose their starch or chloroplasts their chlorophyll and revert to the mitochondrial pro-plastids. Guilliermond relates the occurrence of a second chondriosomal element in green plants to the function of photosynthesis but believes that, although their actual rôle is as yet unknown, the genuine chondriosomes must also play an important part in cell metabolism.

A contrary view to the above was held by Bowen, who believed that mitochondria and pro-plastids are two distinct types of sub-cellular units which can easily be distinguished by their form, structure, reactions to preservatives and stains and behaviour during mitosis. It is probably advisable, therefore, to make no definite pronouncement concerning the relationship of mitochondria and plastids until positive evidence is available.

THE VACUOLAR SYSTEM.—Bounded by a semi-permeable membrane and containing a watery solution of many metabolic products, the vacuoles are conspicuous cytoplasmic inclusions which play an essential part in osmotic phenomena. In the young cells of meristematic tissues, the vacuoles are small and numerous. As the cells differentiate they enlarge and coalesce until eventually one large vacuole, surrounded by a peripheral cytoplasmic layer in which the nucleus lies, occupies almost the whole volume of the cell (fig. 9).

The vacuoles of embryonic cells are difficult to detect, and earlier investigators were led to believe that they arose *de novo* in the course of cell differentiation. This view was lent support by the classical experiment of Pfeffer, who created artificial vacuoles in the plasmodium of *Chondrioderma difforme* by placing it in a saturated solution of asparagin. The plasmodium flowed around the crystals of asparagin, which, by dissolving in the cytoplasm, formed vacuoles indistinguishable from those already there. Pfeffer therefore concluded that every soluble particle in the cytoplasm was the precursor of a vacuole.

De Vries and his student Went, on the other hand, observed the multiplication of vacuoles by fission and believed that they could only arise by division of pre-existing vacuoles.

P. A. and P. Dangeard found in the meristems of the higher plants and in the growing tips of fungal hyphae minute elements in the form of granules or branched filaments which in living tissue stained deeply and evenly with cresyl blue. These closely resembled mitochondria and, as cell differentiation proceeded, were slowly transformed into typical vacuoles by hydration.

Research by Guilliermond into the development of anthocyanin pigments in the teeth of leaflets from the rose-bud, led him at first to believe

that vacuoles were mitochondrial in origin. Later he found that the bodies which seemed to correspond to mitochondria, and were preserved by mitochondrial technique, were in reality minute vacuoles containing tannin with which the anthocyanin was associated.

The development of typical vacuoles from small elements in the meristematic cells can be followed particularly well in the living Barley root stained with neutral red (fig. 9). Mitochondria in the living state are not stained by this stain. Again, by double staining with neutral red and Janus green, Guilliermond was able to trace the simultaneous development of both the mitochondrial and vacuolar systems in the fungus *Endomyces Magnusii*. It seems probable, therefore, that there is no basis for the theory of the mitochondrial origin of vacuoles and that the two systems are quite distinct. How vacuoles do arise, however, is still an unsolved problem. Guilliermond maintains that each colloidal particle secreted by the cytoplasm with a capacity for absorbing water greater than that of the cytoplasm itself is capable of engendering a vacuole. An opposing view is held by Bailey and Zirkle. From observations of the division and distribution between the daughter cells of vacuoles during mitosis in apical meristems they conclude that there is little evidence to support the origin of vacuoles *de novo*. The fact that the solid aleurone grains of seeds are dehydrated vacuoles which regain their fluid state during germination further suggests that the vacuoles are permanent inclusions of the cytoplasm which in one form or another are transmitted from generation to generation.

The problem still awaits a decisive solution.

THE GOLGI MATERIAL.—Many attempts have been made to demonstrate the presence of Golgi material in plants and various cytoplasmic formations have been suggested as its equivalent. Zirkle and other workers using the silver nitrate technique, and Scott (1929) using osmium tetroxide, obtained convincing representations of the Golgi network in plant cells (fig. 10) which they consider simply to be the vacuolar system in its filamentous or reticulate stage. The *osmiophilic platelets* (p. 131), obtained by Bowen, Gatenby and others by treating various plant tissues by osmic methods, are thought by Guilliermond to be in reality vesiculated mitochondria and plastids. The plastid origin of the limosphere in the moss *Polytrichum commune*, and the relationship between the limosphere and the Golgi substance of the animal spermatid (p. 93), leads Weier to believe that plastids of plants correspond to the Golgi material of animals.

After consideration of all the available facts, it seems probable that in the plant cell there is no exact counterpart of the Golgi substance.

CHAPTER V

MITOSIS AND CELL DIVISION

THE term mitosis is sometimes applied to cell division as a whole, but when correctly employed is restricted to the division of the nucleus. *Mitosis*, or *karyokinesis*, is therefore the division of the nucleus to form daughter nuclei. It precedes the division of the cell, or *cytokinesis*, and involves a complicated series of nuclear changes, including the longitudinal doubling of the chromosomes and their separation into two groups. Each daughter chromosome enters a different group and is included in one of the daughter nuclei, and in this way each of the new nuclei receives a set of chromosomes identical with that of the parent nucleus.

Mitosis is sometimes referred to as *indirect nuclear division*. Another method of nuclear division known as *direct division*, or *amitosis*, was formerly believed to be common. In amitosis there are no complicated nuclear or cytoplasmic changes, division being effected by simple constriction of the nucleus and of the cell. It is now known that direct division is of rare occurrence, and even in some of the Protozoa, where division was previously believed to be amitotic, it has been shown that a normal type of mitosis occurs. Amitosis takes place in degenerating cells and in highly specialized cells, and frequently is not followed by division of the cytoplasm.

Mitosis is the usual method of division of the nucleus, but during the maturation of the reproductive cells a special type of mitosis occurs which is called *meiosis* and which involves a reduction of the chromosome number to half that of the somatic cells. Meiosis ensures that the nucleus of the *zygote* receives a half set of chromosomes from the male parent and a half set from the female parent.

At the onset of mitosis the structure of the nucleus, as seen in fixed and stained preparation, undergoes a profound alteration, and structures which constitute the *mitotic* or *karyokinetic figure* become visible. The mitotic figure is made up of the *achromatic figure*, which in animals consists of the centrosomes, astral rays and spindle, and the *chromatic figure* consisting of the chromosomes. The series of changes covering the period from the disappearance of the " resting nucleus " to the reconstitution of the daughter nuclei is divided into four stages. The process is substantially identical in all organisms, the only differences

being the absence of centrosomes and asters in higher plants, and minor details of the structure of the spindle.

THE STAGES OF MITOSIS IN THE ANIMAL CELL

The duration of the mitotic cycle varies from ten minutes to several hours, depending upon the species, the type of tissue, the temperature, and upon other factors.

THE PROPHASE.—It is usual to divide this period into two phases—the *early* and the *late* prophase (figs. 11, b and c, and 12).

In the early prophase, thin thread-like chromosomes become visible in the nucleus. They are frequently coiled, and are slender and stain but lightly with the nuclear dyes. With the high power of the microscope the chromosomes of certain nuclei are visibly composed of a linear series of minute bodies, the *chromomeres*, arranged on a more lightly stained thread (fig. 11, j). During fixation the chromomeres tend to coalesce, so that a single visible granule may be composed of several chromomeres. The chromosomes increase in volume and at the same time shorten and thicken (fig. 11, c). It has been established that the prophase chromosomes are longitudinally double, each having divided in preparation for the subsequent separation of the daughter halves. In some cases the longitudinal split is clearly visible, in others the two halves of the chromosome, or *chromatids*, lie very close together, so that the split cannot be seen.

While these changes are taking place within the nucleus, the *centrosome*, preceded by the division of the *centriole*, divides. The two new centrosomes move apart, small *astral rays* make their appearance in the cytoplasm around each centrosome and a small *central spindle* becomes visible (fig. 11, b).

In the late prophase the centrosomes move further apart, and finally occupy positions close to opposite poles of the nucleus (fig. 11, c). At the same time the astral rays and the spindle increase in size and the chromosomes undergo further contraction. The latter are now deeply stained and, owing to their contraction, the individual chromomeres are no longer distinguishable. In some cases *nucleoli* may persist until the late prophase, in others they disappear at an earlier stage. The *nuclear membrane* now suddenly breaks down, and the chromosomes, each attached to a half-spindle element, take up their positions at the equator of the spindle. The region of attachment is a relatively achromatic part of the chromosome known as the *centromere*. The final stage of the prophase is sometimes referred to as the *prometaphase*.

THE METAPHASE.—The chromosomes are situated at the equator of the spindle to form the *metaphase*, or *equatorial plate* (figs. 11, d, 12, 13 and 14). They are visibly double and lie with the region of the spindle-

attachment in the equatorial plane, so that each daughter chromosome is connected by a half-spindle element with opposite poles of the spindle. The chromosomes may either lie within the spindle or form a ring around the equator.

THE ANAPHASE.—The anaphase is a period of great activity and of relatively short duration. The daughter chromosomes separate and each moves towards an opposite pole of the spindle. In the late anaphase there is an elongation of the middle region of the spindle so that the two groups of chromosomes may be carried beyond the original position of the spindle poles (figs. 11, e, f, 12 and 14).

THE TELOPHASE.—The chromosomes are now more or less closely grouped together (figs. 11, g, 13 and 14); a nuclear membrane appears around each group and two daughter nuclei are formed. The chromosomes become less deeply stained, nucleoli reappear, and each nucleus assumes the character of the " resting nucleus " (fig. 11, h). It is now believed, however, that the chromosomes persist throughout the " resting " period and that fixed material does not give a true picture of nuclear structure. Meanwhile a constriction appears around the equatorial region of the cell. The constriction deepens, and finally the cell divides to form two daughter cells.

When division centres are present mitosis is said to be of the *amphiastral* type. They are absent in many of the Protozoa and in the maturation divisions of the egg, and in these cases an *anastral* spindle is formed.

CELL DIVISION IN PLANTS

The stages of mitosis are similar to those of the animal cell (fig. 15). The time taken to complete the mitotic cycle varies in different types of tissue and is influenced by the temperature and by other factors. Mitosis is typically of the *anastral* type, but in some of the Gymnosperms, Pteridophytes and Bryophytes, central bodies, sometimes surrounded by asters, are present during the division which precedes the formation of the sperm. These bodies act as *blepharoplasts* in the androcyte.

THE PROPHASE.—During the prophase a spindle appears in the cytoplasm and half-spindle fibres arise in relation to the chromosomes. Each chromosome is composed of two chromatids, and in the late prophase their double nature becomes more obvious. In addition to the centromere, secondary constrictions are usually present. In some cases, in preparation for the ensuing mitosis, each chromatid becomes longitudinally double during the late prophase.

THE METAPHASE AND ANAPHASE.—The metaphase and anaphase are similar to the corresponding phases of the animal cell.

THE TELOPHASE.—Nucleoli make their appearance during the reconstitution of the daughter nuclei. In some cases a nucleolus seems to arise

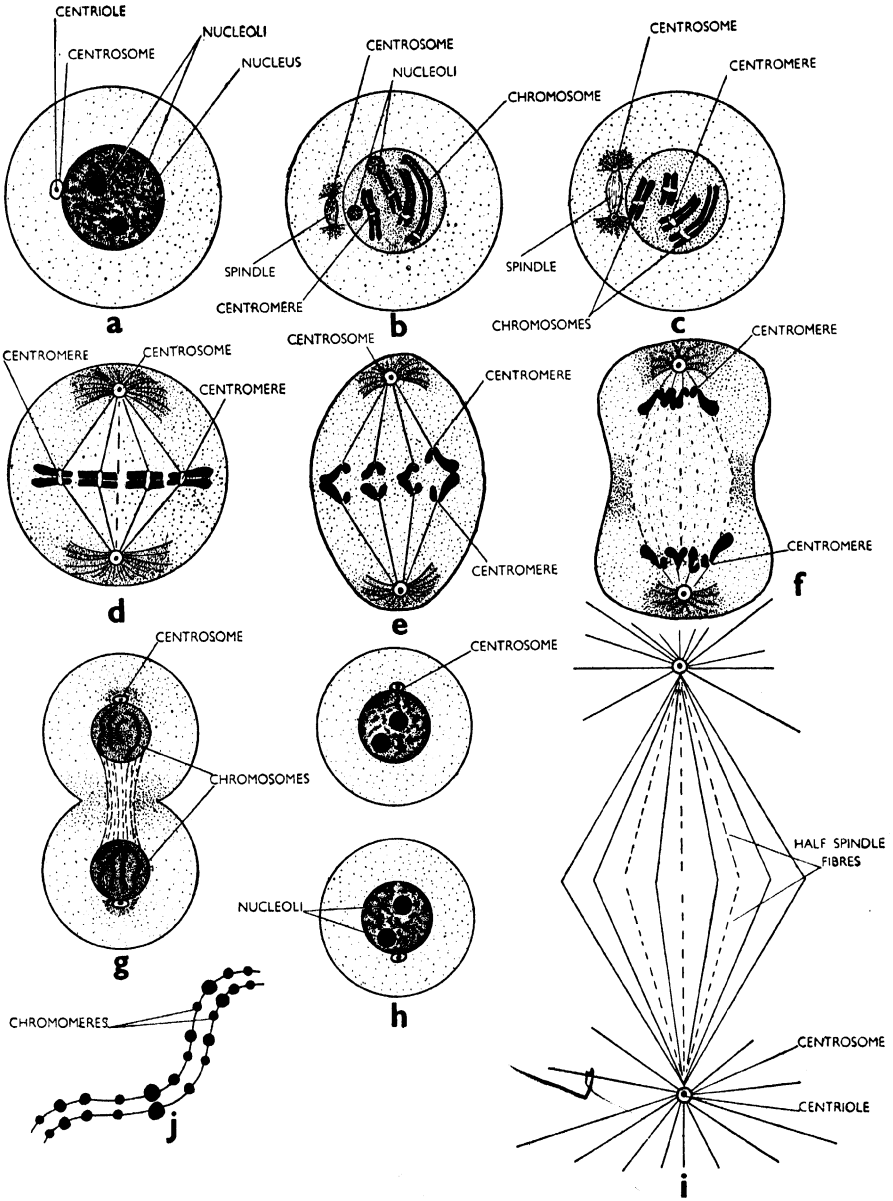


FIG. 11.—Stages of mitosis. Diagrammatic. a, non-dividing nucleus. b and c, early and late prophase. d, metaphase. e and f, early and late anaphase. g, telophase. h, daughter cells. i, achromatic figure. j, prophase chromosome to show arrangement of chromomeres.

in connection with a secondary constriction of a particular chromosome. Such a constriction is known as a *nucleolus organizer*.

THE PHRAGMOPLAST.—In the somatic cells of the higher plants division of the cytoplasm, or *cytokinesis*, is brought about by the formation of a *cell plate*. In the telophase the spindle becomes less distinct in the vicinity of the daughter nuclei and widens in the equatorial region to form a barrel-shaped structure—the *phragmoplast*. The phragmoplast undergoes further widening until it extends completely across the cell. In some cases the cell plate seems to be continuous when first formed, but in fixed material it usually appears to arise as thickenings on the spindle fibres which later form a continuous structure extending laterally to the cell walls. The phragmoplast disappears and the cell plate finally forms the intercellular substance, or middle lamella, upon which cellulose is laid down to form the cell walls of the new cells (p. 23).

Examination of living cells shows that small droplets appear in the equatorial region and unite to form the cell plate. Plasmolysis experiments indicate that the plate is at first composed of fluid, but later is converted into a firm membrane.

THE CHROMOSOMES

CHROMOSOME NUMBER.—The chromosome number is constant for all the somatic cells of an organism and for all the individuals of a given species; there are, however, many exceptions, which will be dealt with later (pp. 119-122). In most animals and in many of the flowering plants the chromosomes are present in pairs; the members of a pair are called *homologous chromosomes*, and where differences of size or shape exist between the pairs it is possible to identify microscopically the members of each pair. It follows that every full somatic, or *diploid*, set is made up of two half, or *haploid*, sets and that the same chromosomes become visible at every somatic mitosis in the individuals of the same species. During the maturation divisions of the germ-cells the diploid number is reduced to the haploid number.

CHEMICAL COMPOSITION.—Chemically the chromosomes are composed of *nucleoproteins*—that is, proteins combined with *nucleic acids*. The nucleic acids are *polymers* of *nucleotides*, and a nucleotide is composed of *phosphoric acid*, a *pentose-sugar* and a *purine* or *pyrimidine base*. Due to the presence of basic groups the chromosomes stain with the nuclear dyes, such as haematoxylin.*

* Another view on the chemical structure of the chromosomes is held by Stedman and Stedman, who claim to have isolated from the nuclei of fish sperm, a histone, desoxyribose nucleic acid, and a new type of protein which forms the principal component of the chromosomes. They believe that at least part of the nucleic acid is external to the chromosomes. (Stedman, E., and Stedman, E. 1943. "Chromosomin, a Protein Constituent of Chromosomes", *Nature*, 152, p. 267.)

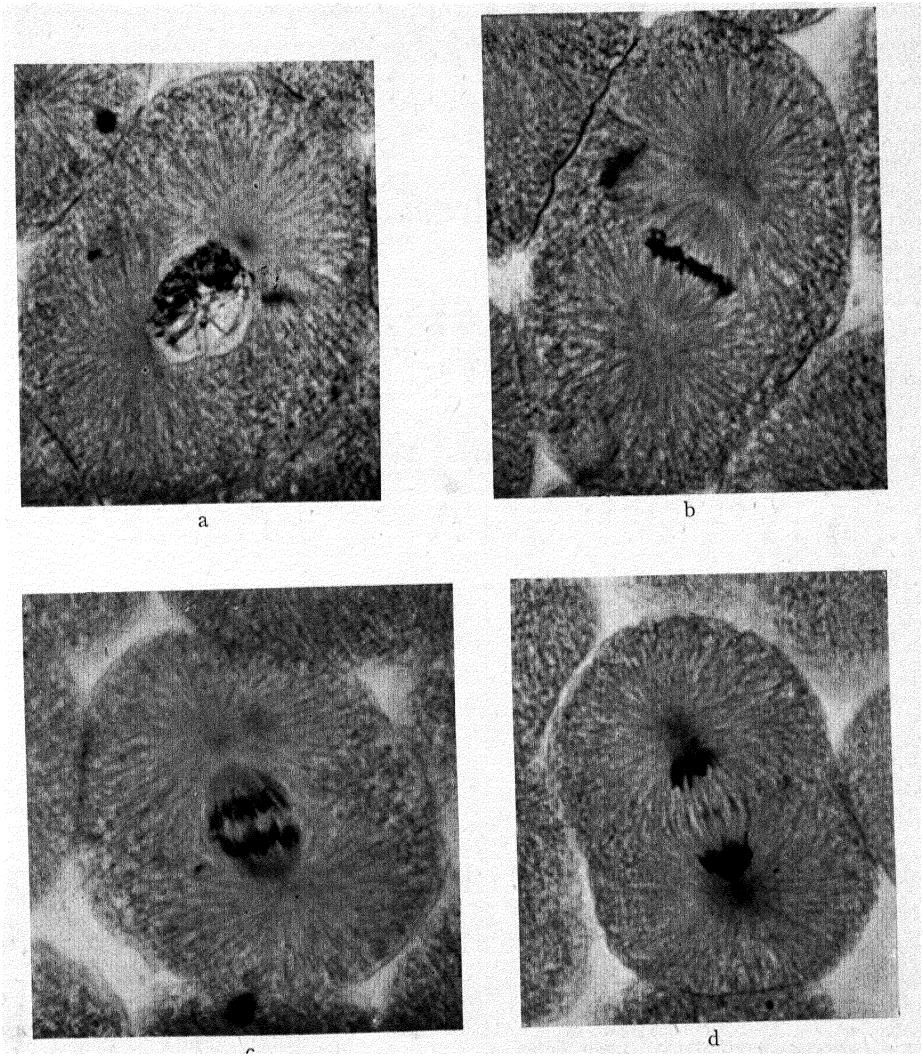


FIG. 12.—Photomicrographs of whitefish blastomeres in mitosis. a prophase. b, metaphase. c. early anaphase. d, late anaphase. × 325.

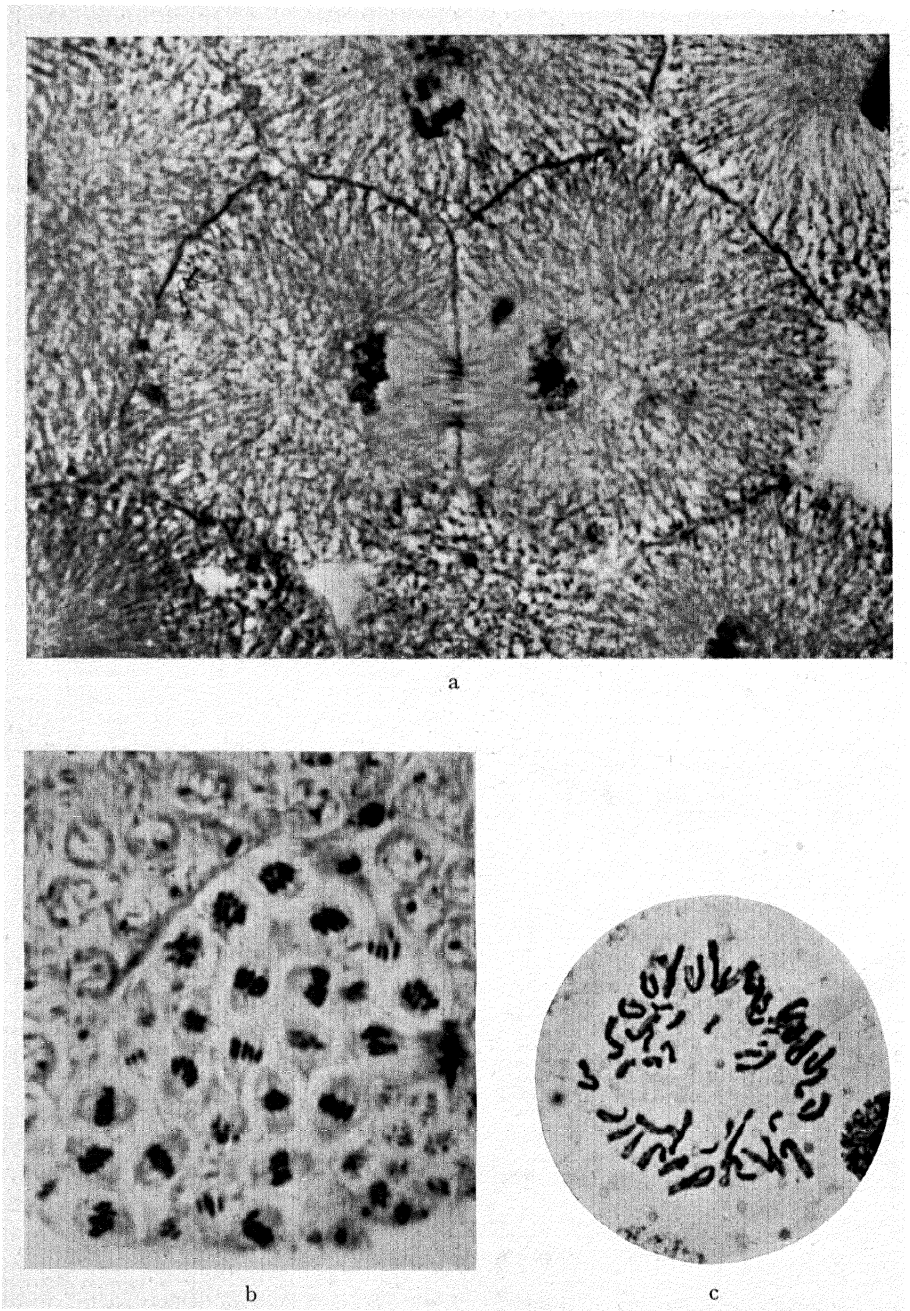


FIG. 13.—Photomicrographs. a, whitefish blastomere in telophase. b, part of testis of *Forficula* showing spermatocytes in metaphase and early anaphase. c, mononucleated cell, human bone marrow. a × 480. b × 340. c × 1230.

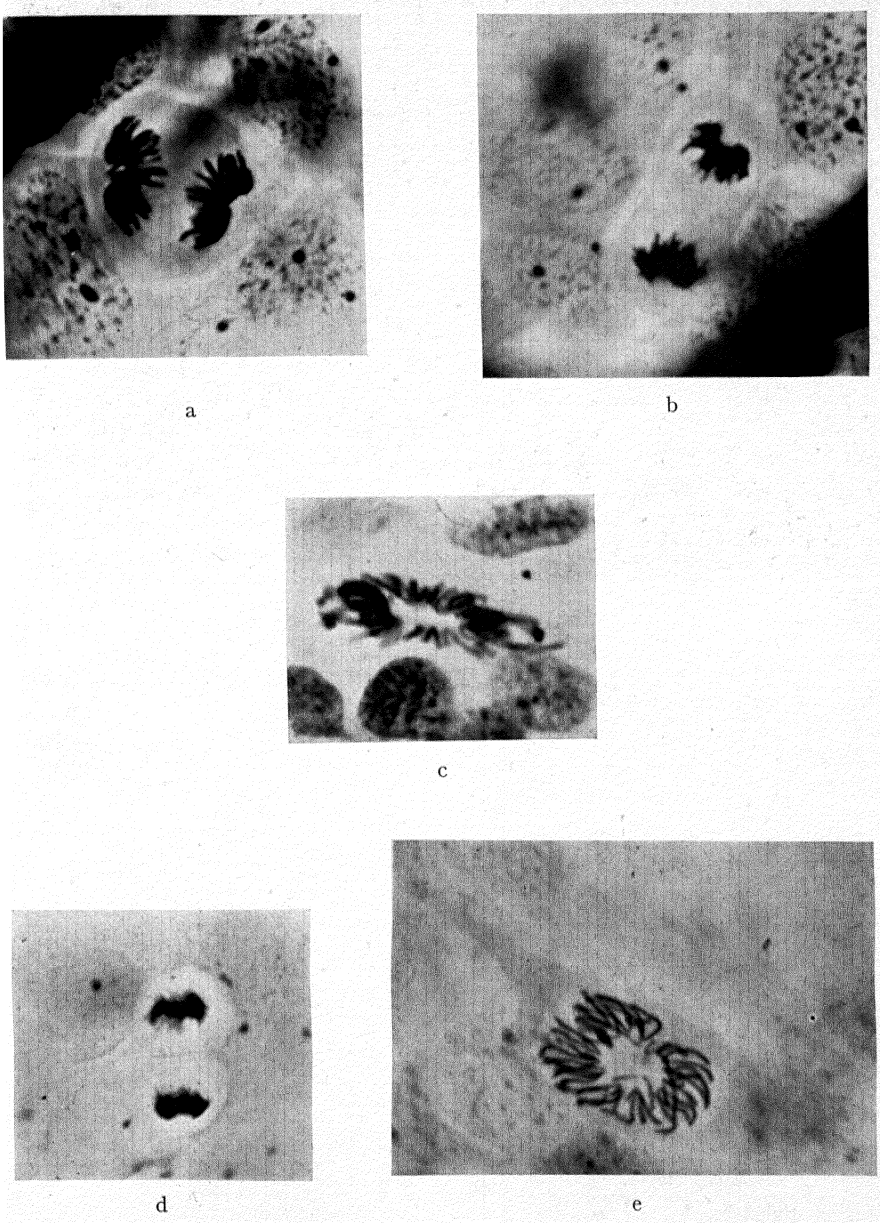


FIG. 14.—Photomicrographs. a–d, connective tissue cells from gill plates of *Ambystoma*. **e,** metaphase plate ; cell from the spinal cord of larva of *Lepidosiren*. × 1050.

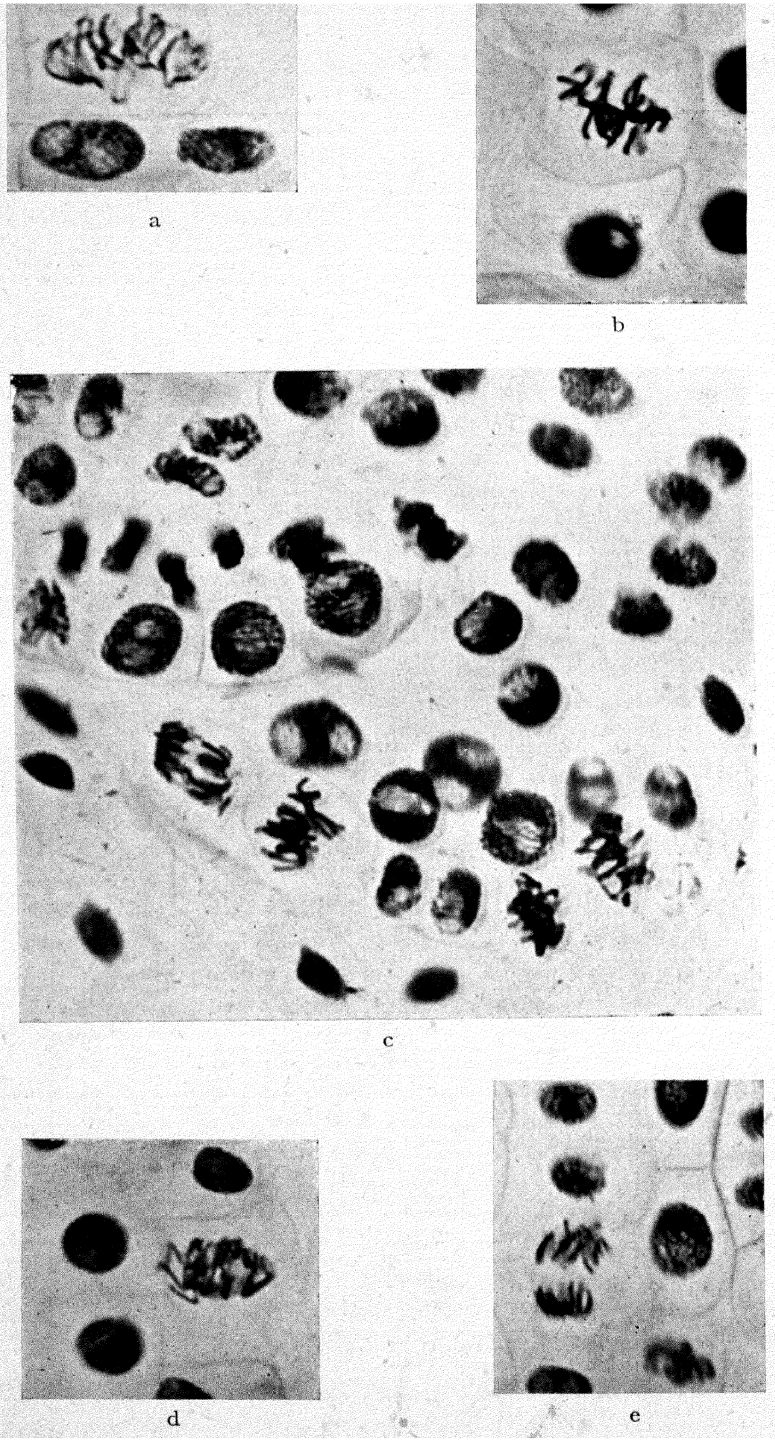


FIG. 15.—Photomicrographs of cells in root-tip of *Alium cepa*. a, prophase. b, metaphase. c, group of cells showing prophase, metaphase and anaphase. d, early anaphase. e, late anaphase.

Darlington (1942 and 1945), reviewing the work of Casperson and others, states that a chromosome consists of a protein fibre, the *chromonema*, in certain regions of which *desoxyribose nucleic acid* is added to form the chromomeres. In the early prophase the chromosomes are visible as long threads of chromomeres arranged in linear series and with *internodes* in between which contain little or no nucleic acid. There is an increase in the amount of nucleic acid in the chromosomes at the time when the nuclear membrane disappears, and the presence of nucleic acid has been demonstrated in the cytoplasm of rapidly dividing cells, but is absent, or present in very small quantities, in cells which have ceased to divide. It is probable, therefore, that nucleic acid is transferred from the cytoplasm to the chromosomes at the onset of mitosis, and that it is passed from the chromosomes to the cytoplasm after each nuclear division. Nucleoli become visible in the telophase and disappear in the prophase; they often arise in connection with a definite region of a chromosome called the *nucleolus organizer*. They contain *ribose nucleic acid*, which is characteristic of the cytoplasm, but it is probable that the two kinds of nucleic acid are mutually convertible, and that the nucleoli play a part in the transference of nucleic acid to and from the chromosomes.

CHROMOSOME CONTINUITY.—As already pointed out, the chromosomes are believed to persist throughout the " resting stage ", but are invisible. It has been suggested that their invisibility is due to their high water content; according to this view they take up water at the end of the mitotic phase and become unfixable. They undergo partial dehydration, and in the prophase become visible as lightly stained threads. They undergo further dehydration, so that fixability increases and reaches its maximum at the metaphase. The chromosomes, therefore, become progressively more deeply stained and compact as the prophase proceeds. It seems unlikely that high water content alone is responsible for the invisibility of the chromosomes during the " resting stage ". The changes consequent upon the transference of nucleic acid may in part be responsible. Nothing, however, is known with certainty regarding the history of the chromosomes during the non-dividing phase.

HETEROPYCNOSIS.—There is sometimes a difference in staining properties between a pair of chromosomes, or a portion of a pair of chromosomes, and the other chromosomes in the nucleus at a certain stage, or stages, of nuclear division. This is known as *heteropycnosis*; it is characteristic of sex chromosomes, but has also been recorded for autosomes. When a chromosome stains more faintly than the others in the same nucleus it is said to be *negatively heteropycnotic*; when it stains more deeply it is said to be *positively heteropycnotic*. Usually only one kind of heteropycnosis occurs in the nuclei of a given organism, but in certain grasshoppers both positive and negative heteropycnosis is exhibited by the chromosomes at different stages in the development of the male germ-cells

MITOTIC DIVISION.—Each prophase chromosome is made up of two longitudinal halves, or chromatids (fig. 11, b, c and j). The longitudinal division is usually not apparent during the early prophase, and there has been considerable controversy as to the stage at which the chromosomes become double. Some workers claim that doubling takes place during the preceding telophase, others that it occurs during the " resting phase ". The chromatids are composed of chromomeres arranged in linear series, and the division of the chromosome is brought about by the doubling of the chromomeres.

SPIRAL STRUCTURE.—It will be remembered that the chromosomes contract and undergo a process of condensation during the prophase; this is due to the chromonema becoming coiled so that it assumes a spiral structure, except at the region of the spindle-attachment, which, consequently, appears as a constriction. By fixing in boiling water, by treatment with ammonia fumes or strong acids, or by squeezing chromosomes under a cover glass, it can be shown that the metaphase chromatids of plants and animals are coiled to form a spiral. There is evidence that coiling is at random and is not constant for a particular chromosome, and that each chromatid of a pair is coiled independently. The direction of coiling may change at the spindle-attachment. During the telophase the chromosomes undergo a process of de-condensation and de-spiralization, and if the " resting stage " is long the spiral structure may be lost before the next prophase. Frequently, however, the early prophase chromosomes are coiled into a loose spiral which is the *relic spiral*, or remains of the metaphase spiral of the preceding division. The relic spiral disappears by the middle of the prophase, and the new spiral begins to develop at the end of this period.

THE CENTROMERE.—The region of the spindle-attachment is known as the *centromere*; its position is constant for a given chromosome and it is more lightly stainable than the other regions. In some organisms a small granule is present in the centromere. The parts of the chromosome on either side of the centromere are called the *arms*. There is a correlation between the position of the centromere and the shape of the chromosome; if attachment is median or sub-median, then the chromosome is U- or V-shaped at the metaphase and anaphase; if sub-terminal, hook-shaped or rod-shaped. Centromeres have not been identified in some animals, and the chromosomes of the germ-track of *Ascaris* possess several spindle-attachments (p. 121).

ANAPHASIC MOVEMENT.—In the early anaphase the centromeres begin to move apart. The arms of the chromosomes remain in contact as the centromere of each daughter chromosome moves towards an opposite pole of the spindle. Finally, the arms separate as if pulled apart (fig. 11, e, and 15, d), and in the late anaphase the central region of the spindle, between the two groups of chromosomes, elongates.

There have been many theories of anaphasic movement. The spindle fibres were thought to represent lines of force; the chromosomes were believed to be drawn to the spindle poles by the traction of the fibres; it has been suggested that the chromosomes are carried by protoplasmic streams, and that movement is brought about by changes of viscosity in the neighbourhood of the chromosomes (Schrader, 1944). Many cytologists now believe that the initial separation of the chromosomes is due to a force of repulsion which operates between the centromeres. This initial movement is followed by the elongation of the central region of the spindle and the consequent further separation of the chromosomes (White, 1937). There appears to be considerable evidence in favour of the acceptance of this view, but other forces may also be involved in the process.

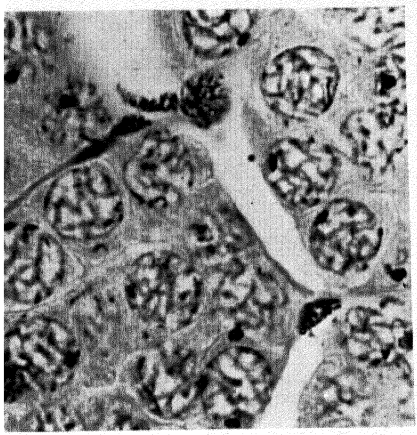
POLYPLOIDY.—The nuclei of somatic cells may contain more than the diploid number of chromosomes, and such cells are said to be *polyploid*. The condition is brought about by multiplication of the chromosomes which is not followed by division of the nucleus. In some cases the diploid group divides to give *tetraploid* nuclei, and in others there is a division of one of the haploid groups resulting in *triploid* nuclei. Further multiplication may take place so that nuclei with an enormous number of chromosomes are produced. Polyploidy does not appear to be widespread among animals but occurs frequently among plants. In some animals and plants all the nuclei, including those of the germ-cells, possess more than two haploid sets of chromosomes, and such organisms are called *polyploid organisms*.

Variation in chromosome number may also be brought about by other means. In *Ascaris equorum* the cells of the germ line contain two large chromosomes, while the somatic nuclei contain numerous small ones produced by the fragmentation of the long chromosomes during the early cleavage stages. The failure of the homologous chromosomes to separate during the reduction division may also lead to variation in chromosome number.

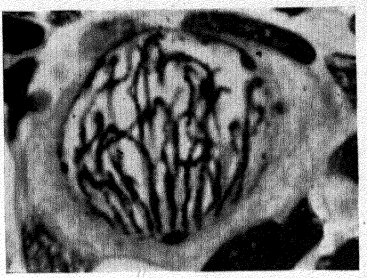
SALIVARY GLAND CHROMOSOMES.—The chromosomes in the nuclei of the salivary gland cells of the Diptera, and also in those of certain other tissues, are in the form of very long thick threads. Each chromosome is closely paired with and wound round its homologue, and if squeezed under a cover glass and suitably stained, is seen to be made up of a number of alternate dark and light transverse bands (fig. 16). The bands of a chromosome vary in size and are constant in position for any one species. The dark bands are rich in nucleic acid, but little of this substance is present in the light areas, or *internodes*. " Chromosome maps " have been made showing the number and disposition of the bands; it is not possible, however, to give the exact total number in a chromosome, as many of the bands are extremely fine and some of the thicker ones may

be made up of finer bands lying very close together. One of the chromosomes in the salivary glands of *Drosophila melanogaster* contains about 1000 bands. As a band is made up of a very large number of granules connected by fine threads with the granules in the next band on either side, it is believed that the salivary gland chromosomes are produced by the repeated division of an original chromosome and the failure of the products to separate. The salivary gland chromosomes are not wound into a tight spiral, and consequently are many times the length of the ordinary somatic chromosomes.

In many species the salivary gland chromosomes lie separate in the nucleus, but in *Drosophila* the more deeply stained, or heterochromatic, regions around the centromeres are fused together to form a structure called the *chromocentre*, to which the nucleolus is attached by a thread. The study of the salivary gland chromosomes has yielded much valuable information regarding chromosome structure



a



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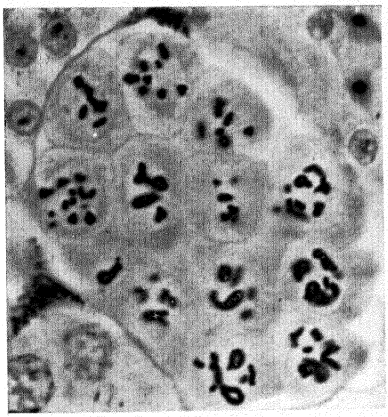

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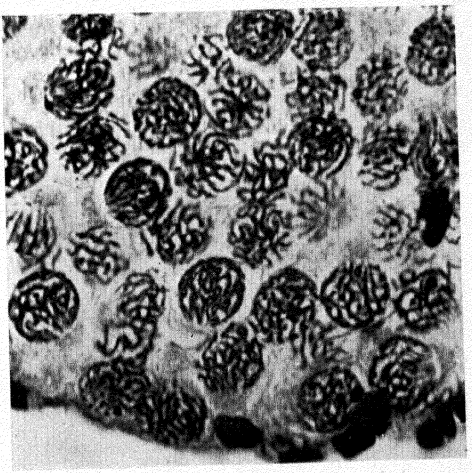


d

FIG. 20.—Photomicrographs. a, primary spermatocytes of *Amphitornus*. × 410. b, oocyte of larva of *Salamandra*. × 950. c, primary spermatocytes of *Amphitornus*. × 305. d, primary spermatocytes of *Batrachoceps attenuatus*. × 410.

CHAPTER VI

MEIOSIS

AT some stage in the development of an animal, cells are set aside from those which form the somatic tissues; these cells give rise to the *primitive*, or *primordial germ-cells*. The series of changes by which the primitive germ-cells are converted into the ripe germ-cells is known as *gametogenesis*.

In the male the primitive germ-cells divide repeatedly and give rise to cells known as *spermatogonia* (fig. 17). The spermatogonia undergo several divisions and finally are transformed into larger cells, called the *primary spermatocytes*. These divide once to form smaller cells—the *secondary spermatocytes*, which, in turn, divide once to form the *spermatids*. The spermatids, without further division, are transformed into *spermatozoa*.

The *somatic*, or *diploid*, number of chromosomes is reduced to the half, or *haploid*, number during the divisions of the spermatocytes, in the course of which the nucleus divides twice but the chromosomes divide only once. The two spermatocyte divisions are known as the first and the second *meiotic divisions*. During the first meiotic division each chromosome divides but the centromeres do not, so that in the anaphase each centromere moves towards a pole of the spindle and carries with it two chromatids. The centromeres divide during the second meiotic division and the chromosomes move apart as in ordinary mitosis; consequently each spermatozoon contains a haploid set of chromosomes.

In the female (fig. 17) the primitive germ-cells give rise to *oogonia* and to certain accessory cells of the ovary. The oogonia are transformed into *primary oocytes*; each primary oocyte divides to give a *secondary oocyte* and a much smaller cell, the *first polar body*, or *polocyte*. The secondary oocyte, by division, gives a *ripe egg*, or *ovum*, and a *second polar body*. The first polar body may divide to form a *third polar body*. The primary oocyte, therefore, gives only one ripe ovum and two or three polar bodies; the latter degenerate. The two polar, or maturation, divisions are the meiotic divisions.

The spermatozoa and ova contain the haploid number of chromosomes only (fig. 18). During fertilization a sperm-nucleus fuses with that of an ovum and the diploid number is restored. It follows that the chromosomes of the zygote-nucleus are made up of two haploid sets, one of which is derived from the male parent and the other from the female parent, and

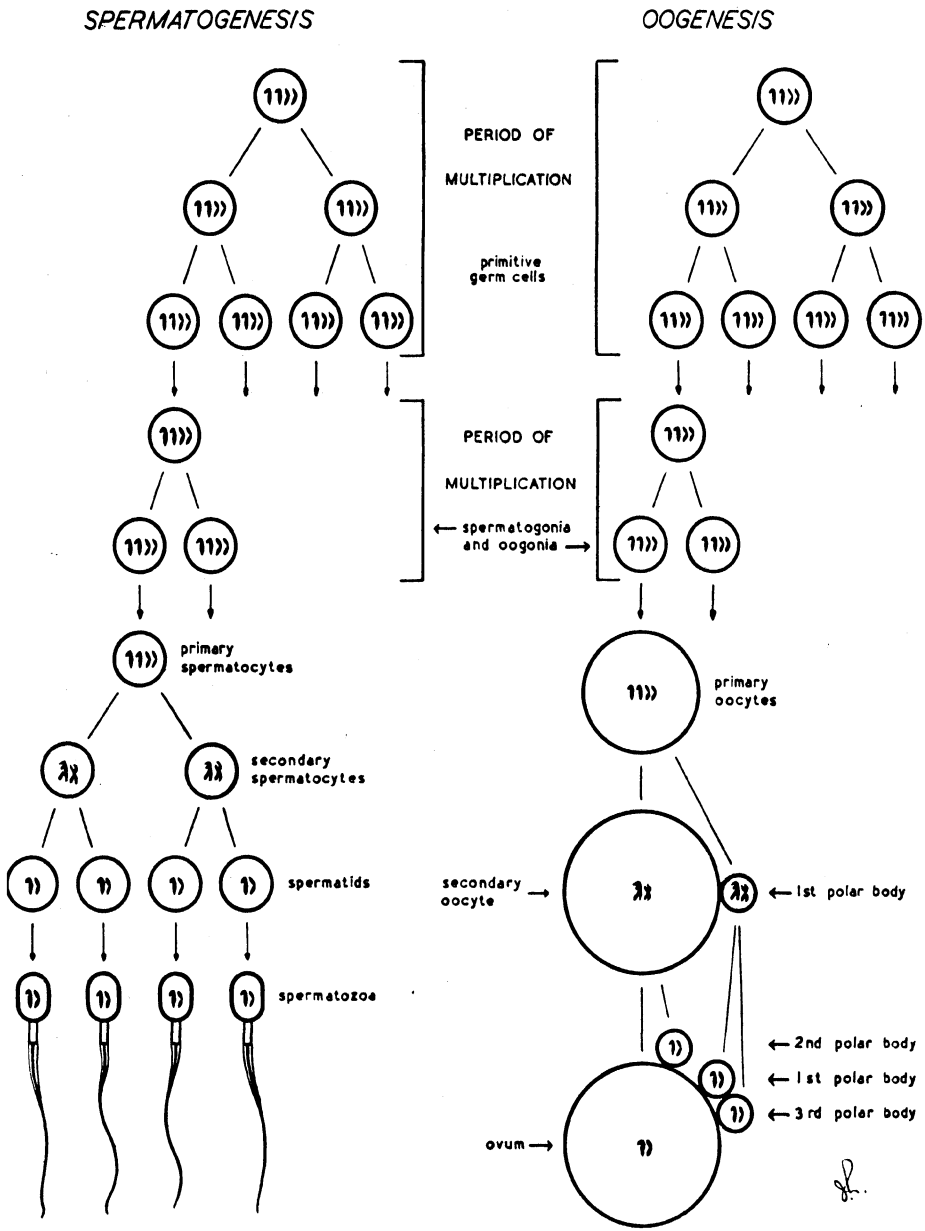


FIG. 17.—Diagram illustrating the stages of spermatogenesis and oogenesis.

that one member of each homologous pair of chromosomes is paternal in origin and the other member maternal in origin. Essential features of gametogenesis and of fertilization are, therefore, the reduction of the diploid number of chromosomes to the haploid and the reconstitution of the diploid group.

The general features of meiosis are closely similar in animals and plants; in parthenogenetic animals and in some other groups, however, modifications of the normal process occur.

THE STAGES OF MEIOSIS

As the prophase of the first meiotic division is complicated and of long duration, it is necessary to divide it into a number of stages (fig. 19). The details of the prophase vary somewhat, but the following account is based on features common to most organisms.

LEPTOTENE STAGE.—Corresponds to the very early prophase of mitosis. The chromosomes are long and probably are not longitudinally double, although some workers believe that division takes place before the leptotene stage. The chromomeres are usually more distinct than during mitosis. The threads are often polarized with the centromeres lying towards one side of the nucleus; frequently they are arranged at random (figs. 19, a, and 20, a).

ZYGOTENE STAGE.—The homologous chromosomes become closely associated in pairs, but fusion between the members of a pair does not take place. When polarized, chromosome pairing begins at the centromeres and extends to other regions, but if not, pairing may begin at any point. Pairing is between homologous chromomeres and is probably brought about by a mutual force of attraction. As pairing takes place the chromosomes contract (figs. 19, b, and 20, b).

PACHYTENE STAGE.—Pairing is now complete, and as the threads are double, or *bivalent*, the apparent chromosome number is reduced to half. The threads wind round each other and shorten and thicken further (figs. 19, c, and 20, d).

DIPLOTENE STAGE.—Each chromosome is longitudinally double, so that each bivalent is composed of four strands, two of which are wound round the other two. As each bivalent chromosome is made up of four chromatids it used to be referred to as a *tetrad*. The attraction between the homologues is now replaced by an attraction between the two chromatids which are formed as the result of the division of each member of a pair (figs. 19, d, and 20, c).

The homologues now tend to repel each other and therefore to separate, but remain held together at certain points known as *chiasmata* (figs. 19, d, e, and 20, c).

DIAKINESIS.—Corresponds to the late prophase of mitosis. The

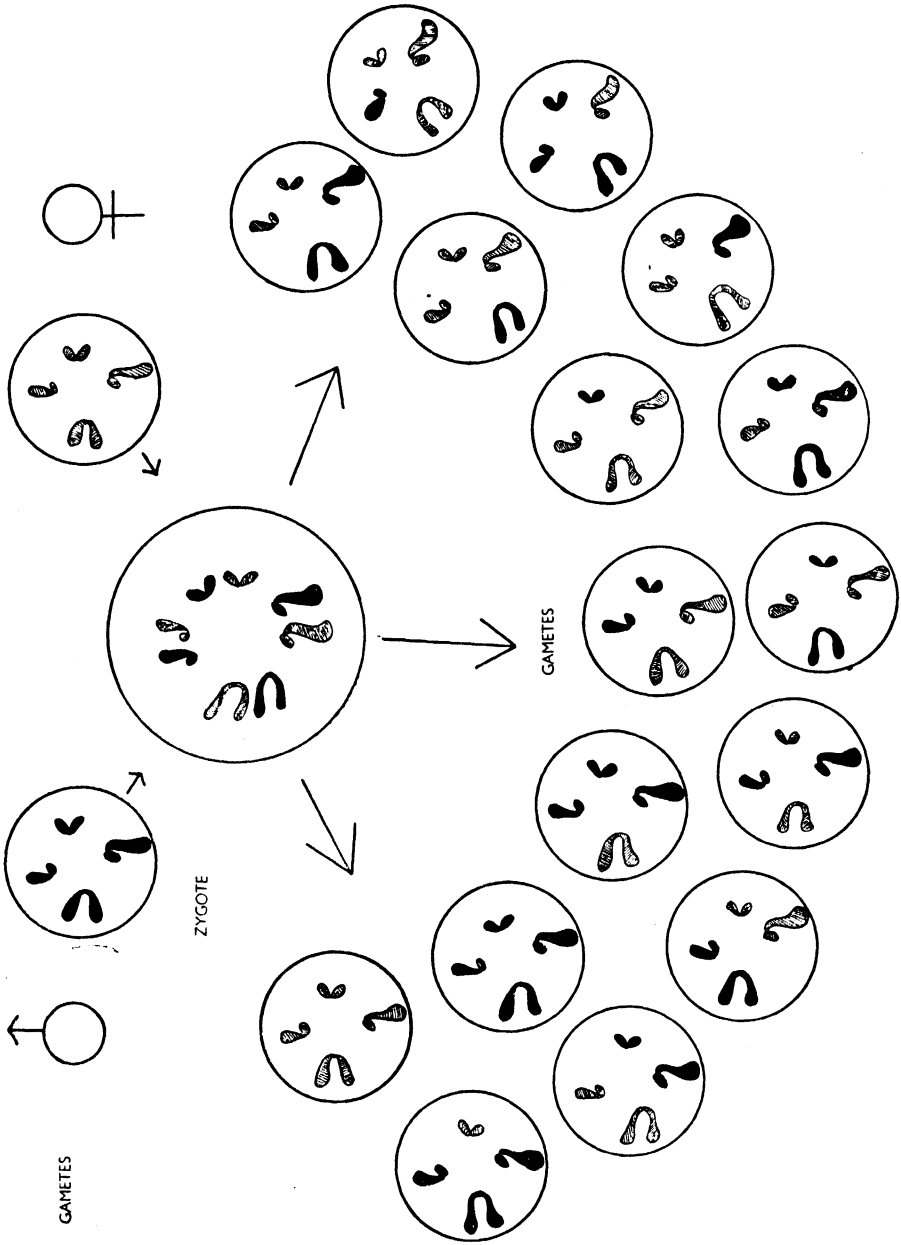


FIG. 18.—Diagram to illustrate the segregation of the chromosomes. Crossing-over and linkage is not represented. Paternal chromosomes black ; maternal chromosomes shaded.

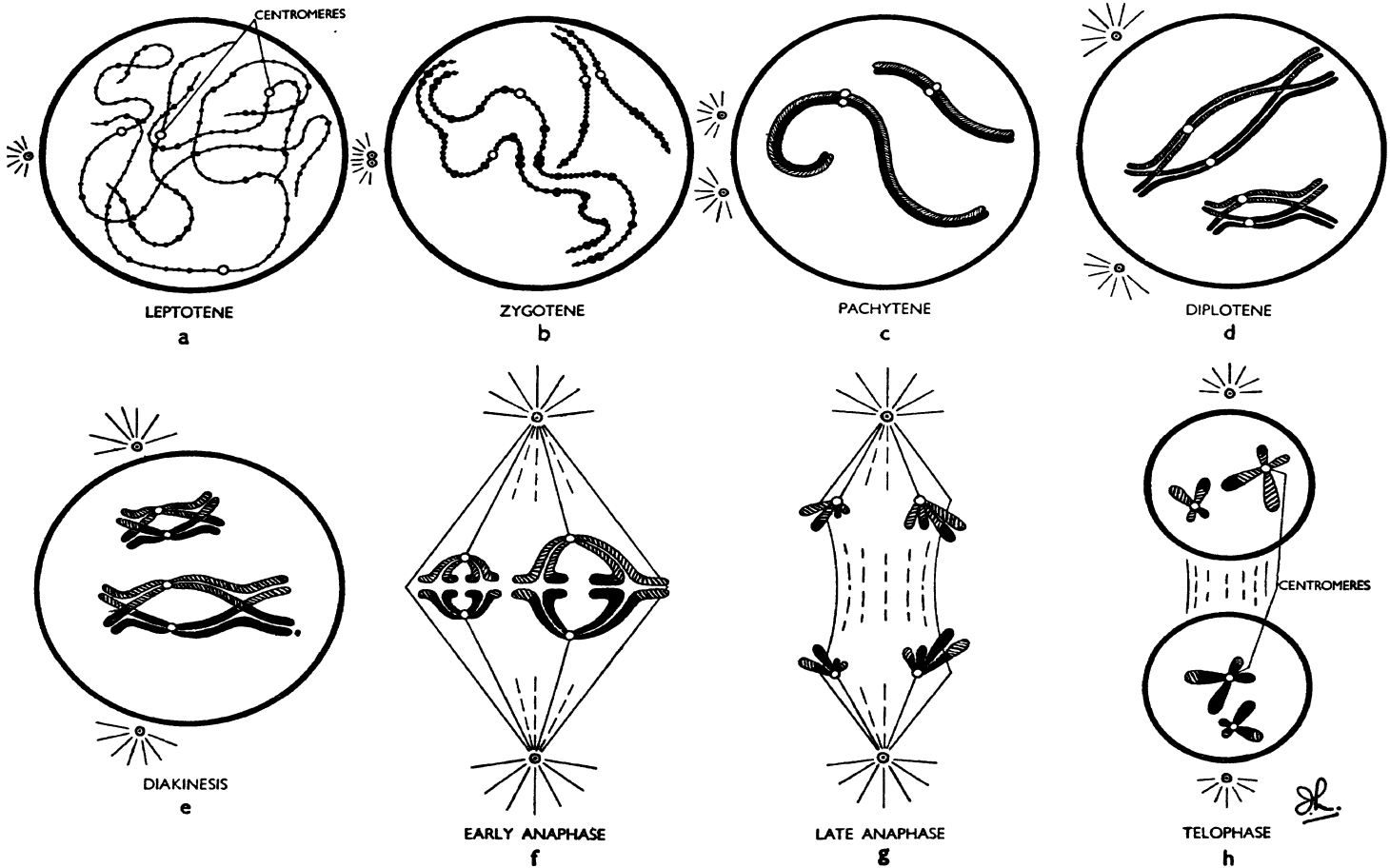


FIG. 19.—Diagram illustrating the stages of meiosis.

bivalents continue to contract by spiralization and tend to lie on the inside of the nuclear membrane as far apart from one another as possible. Each chromatid acquires a double spiral structure in which each gyre of the *major spiral* consists of several turns of the *minor spiral* (fig. 21). The nuclear membrane breaks down and the chromosomes become arranged on the spindle.

METAPHASE.—Each bivalent possesses two centromeres which do not divide as in mitosis, but are arranged equidistant above and below the equatorial plane.

ANAPHASE.—Due to a force of repulsion, the centromeres of each member of an homologous pair move towards opposite poles of the spindle,

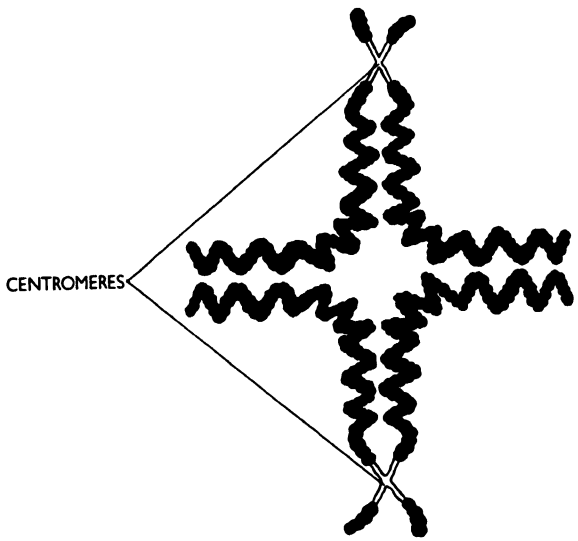


FIG. 21.—Diagrams illustrating the spiral structure of a chromosome. Spiral structure is not usually seen in chromosomes fixed by routine methods; it is shown by special methods which separate the gyres of the spiral. The minor spiral may be in anticipation of the spiral of the second meiotic division. It is possible that a double spiral is present during somatic mitoses. After White, redrawn and slightly modified.

dragging after them the chromatids which have been formed by the division of the chromosomes. In the late anaphase the middle region of the spindle elongates and completes the separation of the two groups of chromosomes (figs. 19, f, g, and 22).

TELOPHASE.—The telophase is similar to that of ordinary mitosis except that each group of chromosomes possesses the haploid number of centromeres (figs. 19, h, and 22).

THE SECOND MEIOTIC DIVISION.—After the first meiotic division the nucleus frequently does not enter upon the " resting stage ". Consequently, the chromosomes may remain compact and do not undergo the

prophase changes. If the interphase is long the chromosomes may become unfixable and pass into the condition of the " resting nucleus ".

The nuclear membrane disappears and the chromosomes take up their position on the spindle ; each is now a *dyad* composed of two chromatids. The chromatids are not approximated throughout their length but are held together at the centromere. In the anaphase they separate along the line of the split which became visible during the prophase of the first meiotic division, and the resulting nuclei contain the haploid number of chromosomes.

CHIASMATA

We have seen that, during the diplotene stage, the chromosomes tend to separate but are held together at certain points—the *chiasmata* ; at least one chiasma is present in each bivalent, except in very few cases, and there may be several. A chiasma is formed by two of the four chromatids breaking at the same level at the end of the pachytene stage and then joining diagonally. In this way an exchange of parts (*crossing-over*) takes place between two chromatids, one of which is derived from the male parent and the other from the female parent.

Owing to the force of repulsion the chromosomes form loops between the chiasmata, so that the diplotene stage frequently has a characteristic appearance (figs. 19, d, and 20, c). In bivalents with a single chiasma two of the arms rotate through an angle of about 180°. In bivalents with several chiasmata the rotation is usually through an angle of about 90°, so that successive loops between the chiasmata lie in planes at right angles to one another.

The chromosomes which separate in the anaphase of the first meiotic division are not identical with those which paired in the zygotene stage. As the result of the breaks which occur during the pachytene stage, at the same level in two of the four chromatids of a bivalent, an interchange of parts takes place between the paternal and the maternal chromosomes. The anaphase chromosomes are, therefore, new combinations of the chromosomes which paired in the zygotene stage (fig. 19, f and g), but between the centromere and the first chiasma on either side two paternal chromatids separate from two maternal chromatids.

It seems probable that the break in a chromatid is brought about by a localized strain set up in the spirally twisted chromosomes. As the result of the break the strain is diminished in the adjacent region of the chromosome, and consequently crossing-over does not take place for some distance on either side of a chiasma. This is known as *interference*.

The average number of chiasmata in a bivalent is called the *chiasma frequency*. If on the average there is one chiasma in a bivalent, then that bivalent is said to have a chiasma frequency of 1·0. In some organisms crossing-over is restricted to certain regions, but more often

it may take place in any region of a chromosome. The relationship between two adjacent chiasmata varies. The two chromatids of the first chiasma may enter into the formation of the second chiasma; the second chiasma may involve only one of the chromatids of the first, or may be formed by the two chromatids which did not enter into the formation of the first chiasma.

TERMINALIZATION

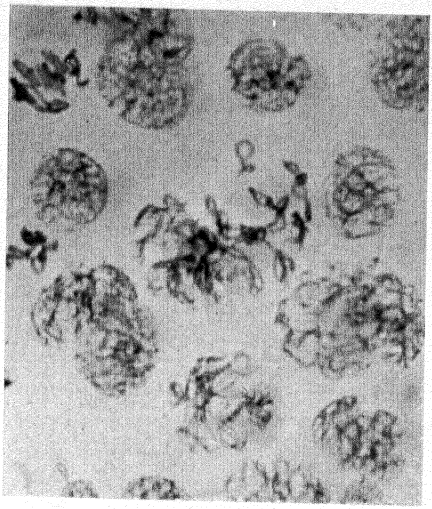
As the loops open out the chiasmata move towards the ends of the bivalent; the movement may be slight or all the chiasmata may move to the ends of the chromosomes. In the latter case the four chromatids remain in contact at their ends. Terminalization is due to the force of repulsion being greatest inside the closed loops; it may continue into the metaphase.

During the anaphase a force of repulsion operates between the centromeres, and if terminalization has taken place, the ends of the chromosomes are torn apart. If terminalization has not already taken place, the chiasmata move away from the spindle-attachments and slip off at the ends of the chromosomes (fig. 23).

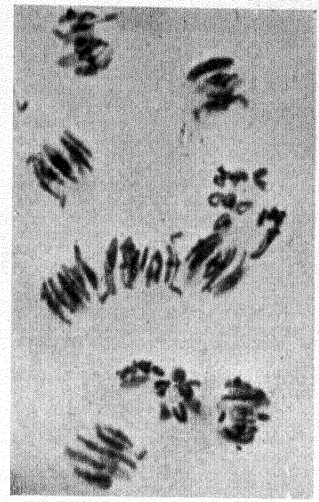
MEIOTIC PAIRING

The pairing of the homologous chromosomes is due to a force of attraction which operates between homologous chromomeres. That the homologous chromomeres pair can be demonstrated in chromosomes in which the chromomeres are distinct, and is also shown by the behaviour of inverted regions where a part of a chromosome becomes reversed. If the inverted region is short it remains unpaired and forms a loop; if a longer portion is inverted the loop twists round so that the homologous chromomeres pair (fig. 24). Additional evidence has been produced by the study of chromosomes, in which a short region has been lost; here the corresponding region of the homologous chromosome forms an unpaired loop.

A force of repulsion exists between the surfaces of somatic chromosomes, and during the prophase the threads usually lie at random and do not come into contact with one another. In certain somatic nuclei, particularly in the Diptera, due to a mutual force of attraction which is greater than the force of repulsion, the homologous chromosomes tend to lie together. Meiotic pairing may be explained in the following way. During the zygotene and pachytene stages the attraction force between the members of an homologous pair of chromosomes is greater than, or replaces, the force of surface repulsion. In the diplotene stage the attraction force between the homologous chromosomes ceases, and is replaced by a force of attraction between the two chromatids which have



a



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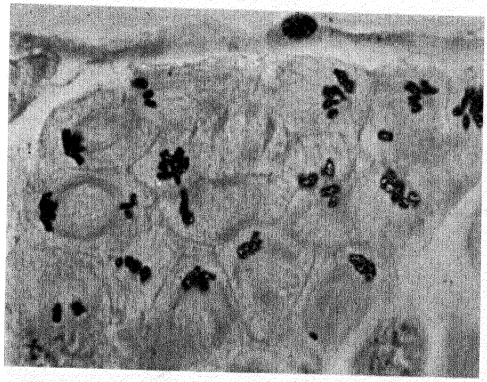

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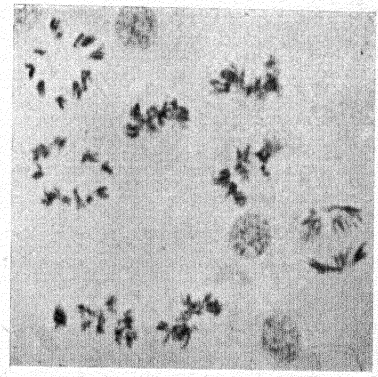


d

FIG. 22.—Photomicrographs. a, *Triton vulgaris* ; prophase. b, *Amphitornus* ; telophase. c, *Triton vulgaris* ; metaphase and early anaphase. d, *Triton vulgaris* ; metaphase and anaphase. × 485.

resulted from the division of each chromosome. At the same time a force of repulsion again operates between the homologous chromosomes, so that they separate but are held together at the chiasmata. Terminalization takes place, and in the anaphase the chromosomes move apart owing to the force of repulsion between the centromeres.

A recent explanation of meiosis is based upon the assumption that there is an alteration of nucleic acid metabolism (Darlington, 1942 and 1945). According to this view, at the onset of the first meiotic division, nucleic acid is deposited on the chromomeres before their reproduc-

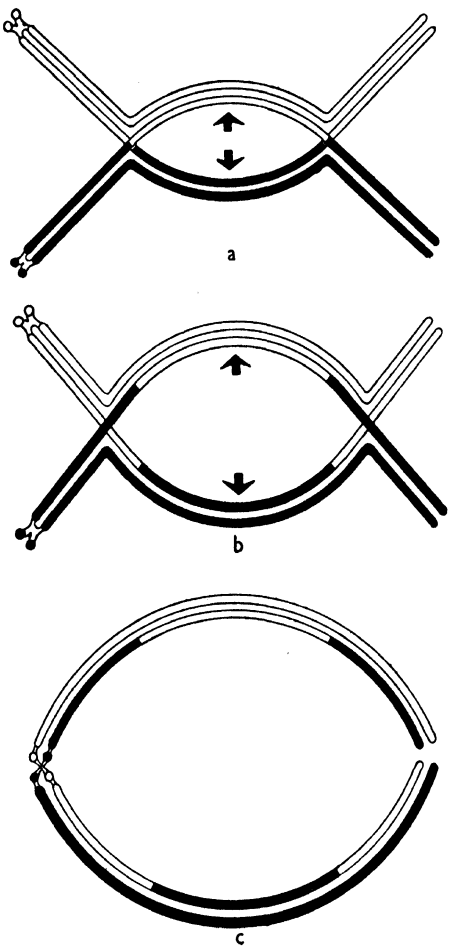


FIG. 23.—Diagram illustrating terminalization of two chiasmata. a, early diplotene when the chiasmata correspond in position to the points of crossing-over. b, early diplotene. c, diakinesis. After White, redrawn and slightly modified.

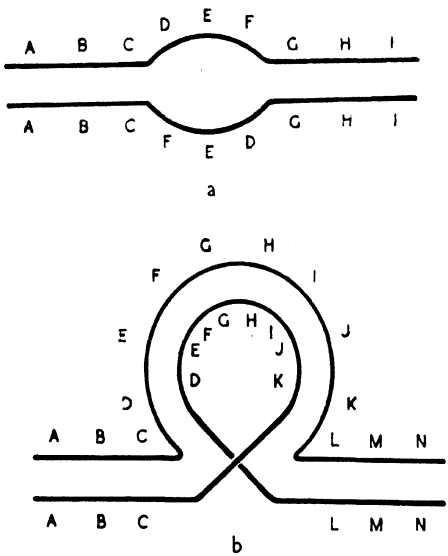


FIG. 24.—Diagrams illustrating inversion. a, short inverted region. b, longer inverted region.

tion. The chromomeres of a prophase thread are consequently single, and the attraction which they possess for similar materials is satisfied by pairing with the chromomeres of the homologous chromosome. (In mitosis this attraction is between the homologous chromomeres of the divided chromosome.) The paired threads continue to receive nucleic acid,

become spiralized and coil round each other. Later, the threads become double and repel each other. Owing to the strain set up by coiling, the chromosome threads break and form chiasmata.

GENES

The factors which determine the hereditary characters of an organism are carried by the chromosomes. These units of heredity are called *genes*; they are arranged in the chromosomes in linear series and may be identical with the chromomeres. As the distribution of the chromosomes during the first meiotic division is at random, depending upon the position which the members of an homologous pair take up on the spindle at the metaphase, it follows that all possible combinations of the chromosomes are present in the ripe germ-cells (fig. 18). The *segregation* of the chromosomes is an important factor in the distribution of the genes among the ova and the spermatozoa. Crossing-over leads to new combinations of the paternal and the maternal chromosomes.

CHAPTER VII

GAMETOGENESIS IN ANIMALS

IN most animals the sexes are separate and germ-cells of the same kind, either spermatozoa or ova, are produced in the gonads of an individual. Hermaphroditism occurs normally in many invertebrates, fish and Amphibia, and has been recorded as an abnormality in all classes of the Vertebrata. Separate testes and ovaries may be present in hermaphroditic animals, or sperms and ova may be produced in the same gonad.

During gametogenesis the germ-cells undergo differentiation, and in the male are converted into highly specialized and active cells—the spermatozoa—and in the female into relatively very much larger, less specialized and inactive cells—the ova. The sperm-forming cells lose most of their cytoplasm so that the ripe sperm consists of a nucleus and usually a long tail, some of which is covered with an extremely thin sheath of cytoplasm containing certain cytoplasmic components. The cytoplasm of the egg, on the other hand, increases in amount, and in the mature ovum contains nutritive material which constitutes the *yolk* or *deutoplasm*.

THE OVUM

An egg-cell in which growth has taken place and which has undergone the two maturation divisions is known as the *ovum* (fig. 17). The yolk may consist of a few globules or granules, but in some animals it almost completely fills the cell so that the active cytoplasm is restricted to a small area surrounding the nucleus. It may be either fatty or protein in nature, and in the eggs of some animals both types are present. Golgi elements and mitochondria are present in the ovum.

The nucleus of the primary oocyte undergoes the early prophase changes of the first maturation division (p. 39); the chromosomes become invisible, the nucleus becomes large and vesicular and is known as the *germinal vesicle*; the cell increases in size and yolk is formed. Maturation may occur within the ovary, but in many animals the second meiotic division, and sometimes the first meiotic division, takes place after the egg is discharged from the ovary. The egg-nucleus is re-formed after the second division; it is smaller than the germinal vesicle and is now called

the *female pronucleus*. Each polar body contains a nucleus and a small amount of cytoplasm together with a few Golgi elements and mitochondria.

The ovum is surrounded by a thin membrane which is called the *vitelline membrane*, and on fertilization a membrane, known as the *fertilization membrane*, separates from the surface of the egg. It was formerly thought that the fertilization membrane is a new structure, but there is reason to believe that it is the vitelline membrane. A thicker membrane lies inside the vitelline membrane; it sometimes shows radial striations and consequently is known as the *zona radiata*. A secondary envelope is often secreted by the follicle-cells, and in insects is composed of a substance related to chitin and is called the *chorion*. In the eggs of many animals protective envelopes are also present, such as albumen, the shell membrane and shell of reptiles and birds, and the egg capsules of fish and certain invertebrates. These are formed as secretions of the uterus or of the oviduct.

The cells which arise from the fertilized ovum undergo differentiation to form the tissues of the new individual, and during adult life cells which have retained their unspecialized embryonic character divide, become differentiated, and replace older cells which are lost or have degenerated. The history of the germ-cells illustrates cellular differentiation, but this process is common to all types of cells.

OOGENESIS

The mammalian ovary is chosen to illustrate ovarian structure. It is surrounded by the *germinal epithelium* and is divided into a cortical and a medullary region (fig. 25, a). The *cortex* contains oocytes in various stages of development, follicle-cells which are protective and probably have a nutritive function, connective tissue, and interstitial cells which are secretory in function. The *medulla* contains connective tissue, blood vessels and lymph spaces.

The germinal epithelium gives rise to the *oogonia* (fig. 17), which are proliferated early in life and are, apparently, not formed after sexual maturity. The *primary oocytes* at first lie close to the germinal epithelium: later they increase in size, sink into the deeper parts of the cortex, and become surrounded by a single layer of *follicle-cells*. With the further growth of the oocyte the follicle-cells become many layers thick, and are surrounded by the *theca*, which in the older follicles is differentiated into the cellular *theca interna* and the fibrous *theca externa*. The connective tissue, or *stroma cells*, are situated outside the theca and separate the follicles from one another; the *interstitial cells* are present amongst the cells of the stroma. Later, a cavity, filled with fluid and known as

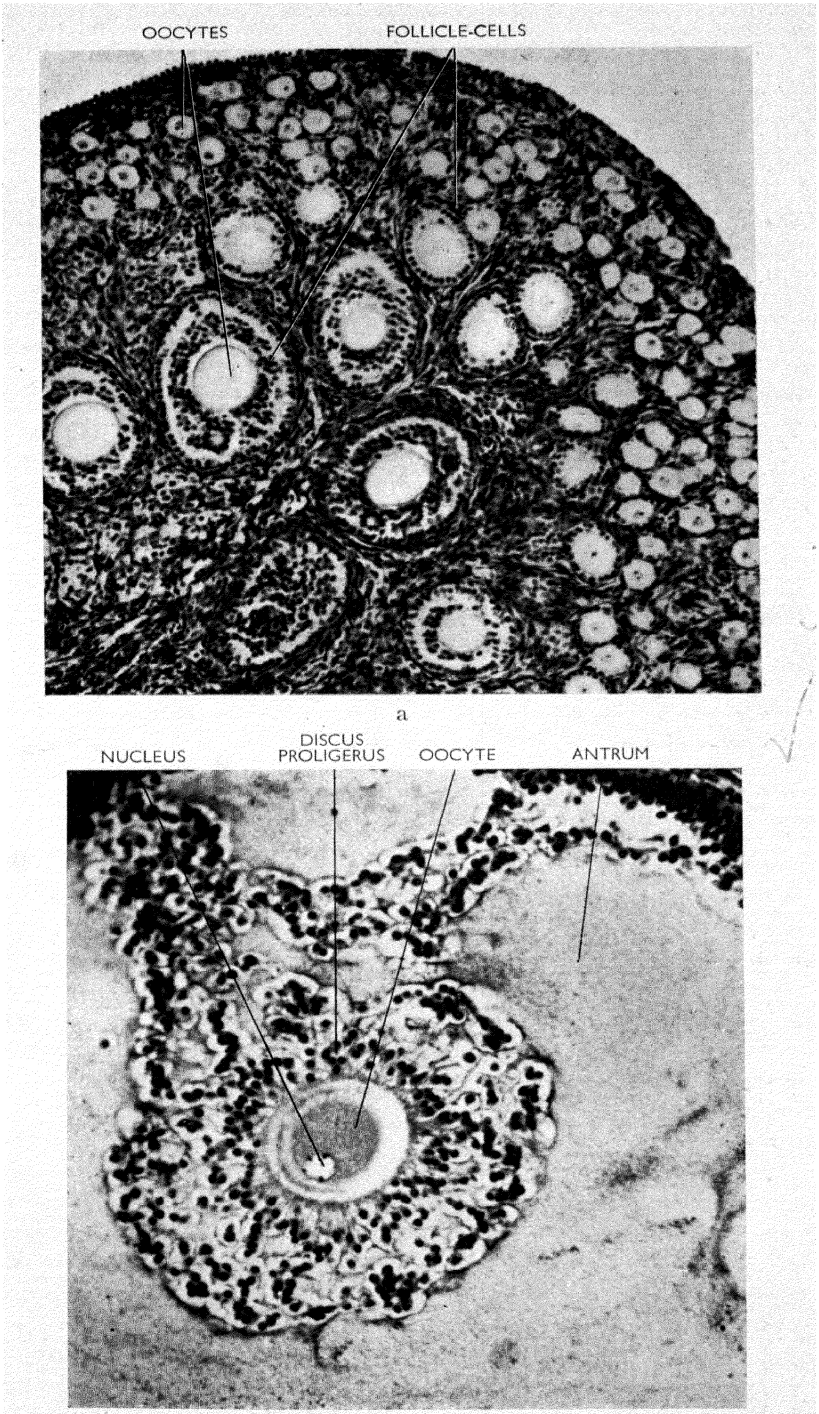


FIG. 25.—Photomicrographs of ovary of rabbit. a, part of ovary of young animal showing oocytes in various stages of development. × 80. b, part of Graafian follicle of older animal showing an oocyte surrounded by the cells of the *discus proligerus*. × 180.

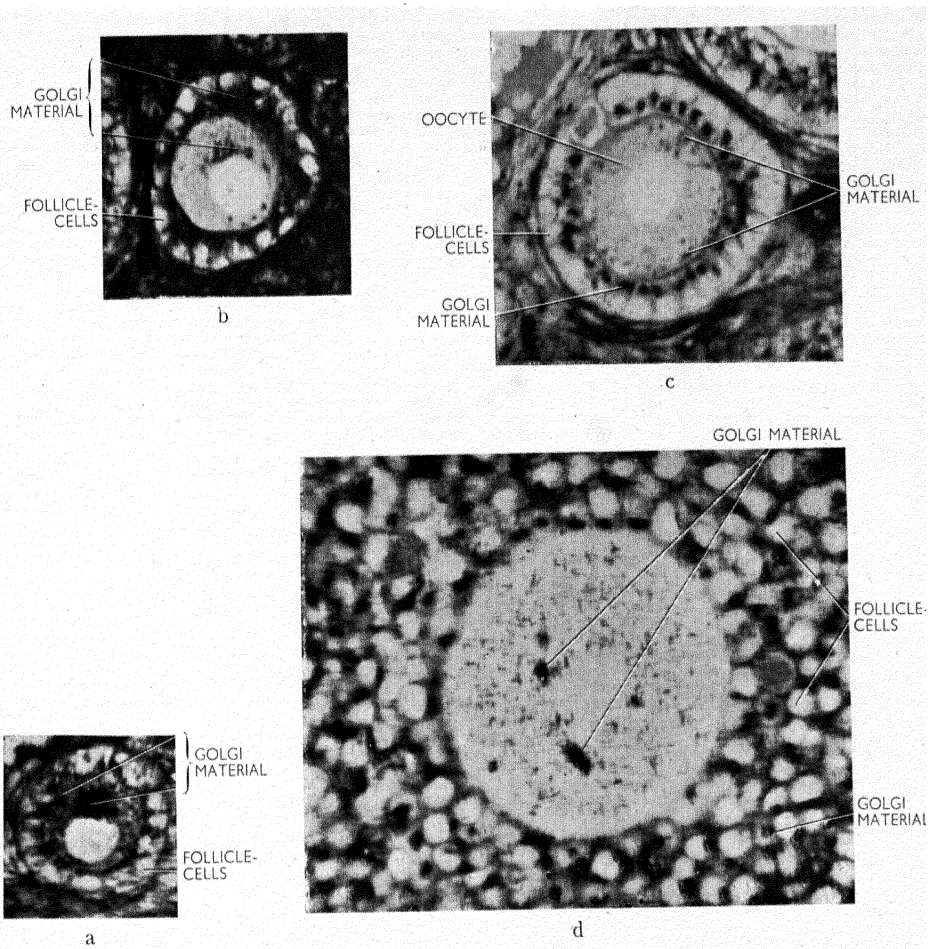


FIG. 26.—Photomicrographs of oocytes and follicle-cells of mouse. a, young oocyte surrounded by a single layer of follicle-cells. The Golgi material is localized at one pole of the nucleus. b, slightly older oocyte. The Golgi elements are beginning to scatter through the cytoplasm. c, slightly older oocyte. The Golgi elements are distributed through the cell. d, later stage. Most of the mitochondria form small clumps. The Golgi elements are scattered through the cytoplasm, but some are in contact with the clumps of mitochondria. The Golgi material of the follicle-cells is localized at one pole of the nucleus. × 430.

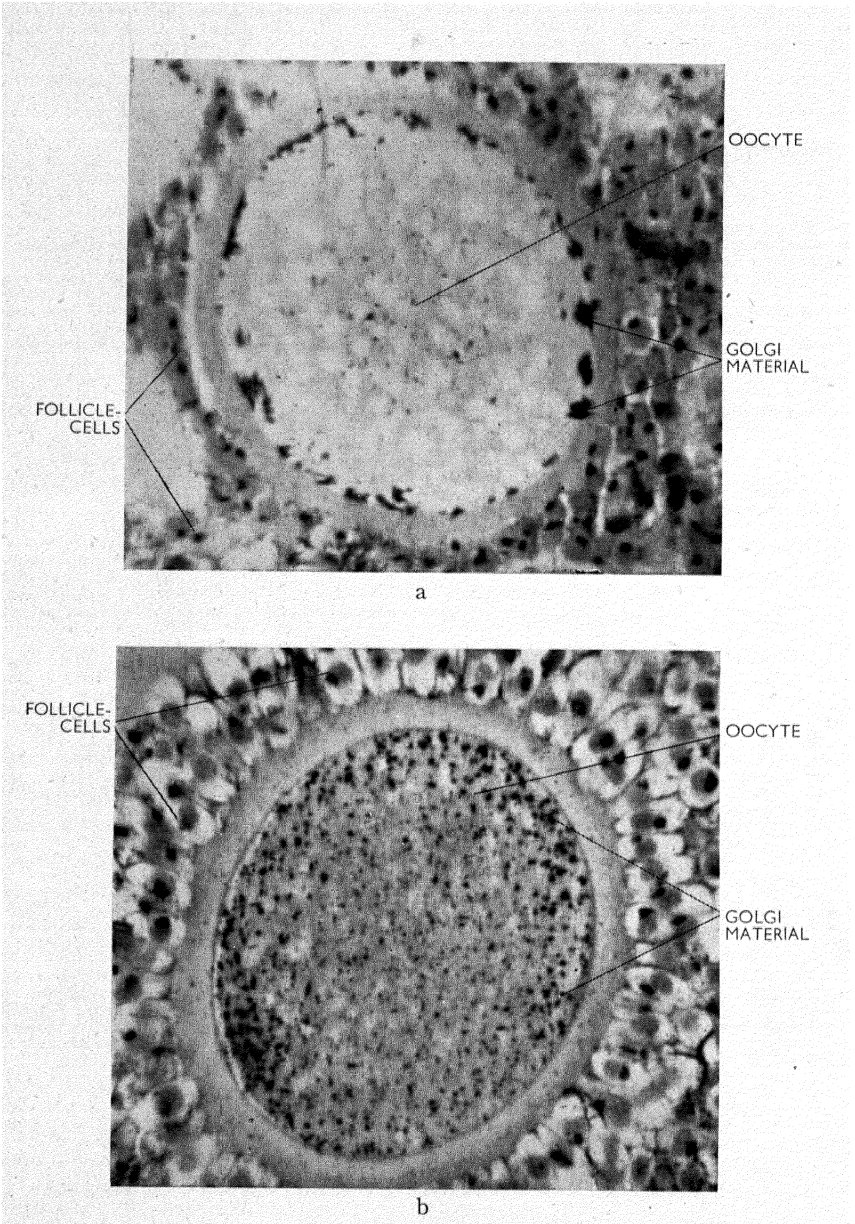


FIG. 27.—Photomicrographs of oocytes and follicle-cells of rabbit. When the Golgi material spreads out from its localized position it becomes concentrated at the periphery of the oocyte. Later, the elements are distributed through the cytoplasm. (Personal communication from Dr. I. Zlotnik). a, oocyte with the Golgi material in the peripheral cytoplasm. b, oocyte in Graafian follicle ; the Golgi elements are scattered through the cell. The Golgi material of some of the follicle-cells is shown localized at one pole of the nucleus. × 425.

the *antrum*, appears in the follicle. The oocyte, surrounded by some of the follicle-cells which form the *discus proligerus*, projects into the cavity, and the remaining follicle-cells form the *membrana granulosa*. The whole follicle is now called a *Graafian follicle* (fig. 25, b). With the further accumulation of liquid the follicle ruptures, and the ovum, together with liquid and cells of the discus proligerus, is discharged from the ovary. The sperm enters the egg and maturation is completed within the oviduct. The ruptured follicle is now transformed into an organ with endocrine functions called the *corpus luteum*, the cells of which are formed from the membrana granulosa and from the theca interna. In unmated females the corpora lutea undergo regression before the next ovulation, but in pregnant animals they persist and produce secretions which inhibit oestrus, bring about changes in the uterine mucosa and affect the development of the mammary glands.

Owing to the small number of yolk globules present in the oocyte, the mouse is a convenient subject in which to follow the behaviour of the cytoplasmic components during the stages of oogenesis (fig. 26). In this animal there is an embryonic proliferation of cells from the germinal epithelium; later, these cells degenerate. A second proliferation begins from one to two days after birth, continues almost to sexual maturity, and gives rise to the ova. In early oocytes which have not yet acquired a follicle-wall, and in those in which a single layer of follicle-cells has just been formed, the Golgi material is localized at one side of the nucleus. The Golgi material is made up of rods and granules which closely invest the *archoplasm*, or substance which surrounds the centrosome during the interphase. At this stage the mitochondria are granular and are scattered in the cytoplasm round the nucleus and Golgi material. Shortly after a single layer of follicle-cells has been formed, the localized Golgi material begins to spread out, and at a slightly later stage the elements are distributed throughout the cell. The mitochondria scatter through the cytoplasm and are present in the peripheral as well as the central regions. In oocytes surrounded by several layers of follicle-cells, and also in those situated in mature follicles, the mitochondria are for the most part collected into small clumps. The Golgi elements, which are now granular in appearance, tend to lie in contact with the clumps of mitochondria. Fat globules are not present in the mouse egg, and albuminous yolk is restricted to a few globules lying inside the cell membrane.

Several *nucleoli* are visible in the early oocytes, but in the older cells one or two only are present. They become irregular in outline and small bodies, or buds, appear to migrate from their surface into the nucleoplasm. Later, the buds are present inside the nuclear membrane and similar bodies make their appearance in the cytoplasm, at first close to the nuclear membrane but later through the cell generally. These cytoplasmic bodies disappear and the yolk globules increase in size. It has been suggested

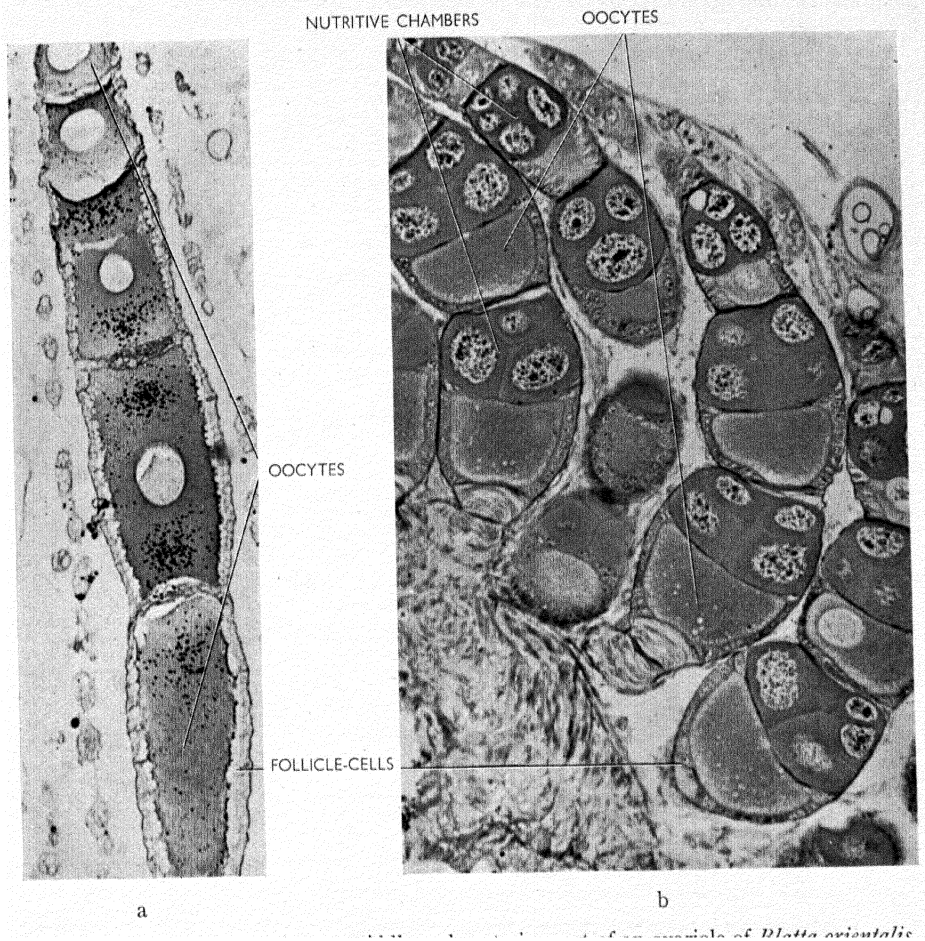


FIG. 29. Photomicrographs. a, middle and posterior part of an ovariole of *Blatta orientalis*. There are no nutritive chambers. Yolk globules are present in the larger oocytes. b, middle and posterior parts of ovarioles of *Stenophylax stellatus*. Nutritive chambers are present. × 100.

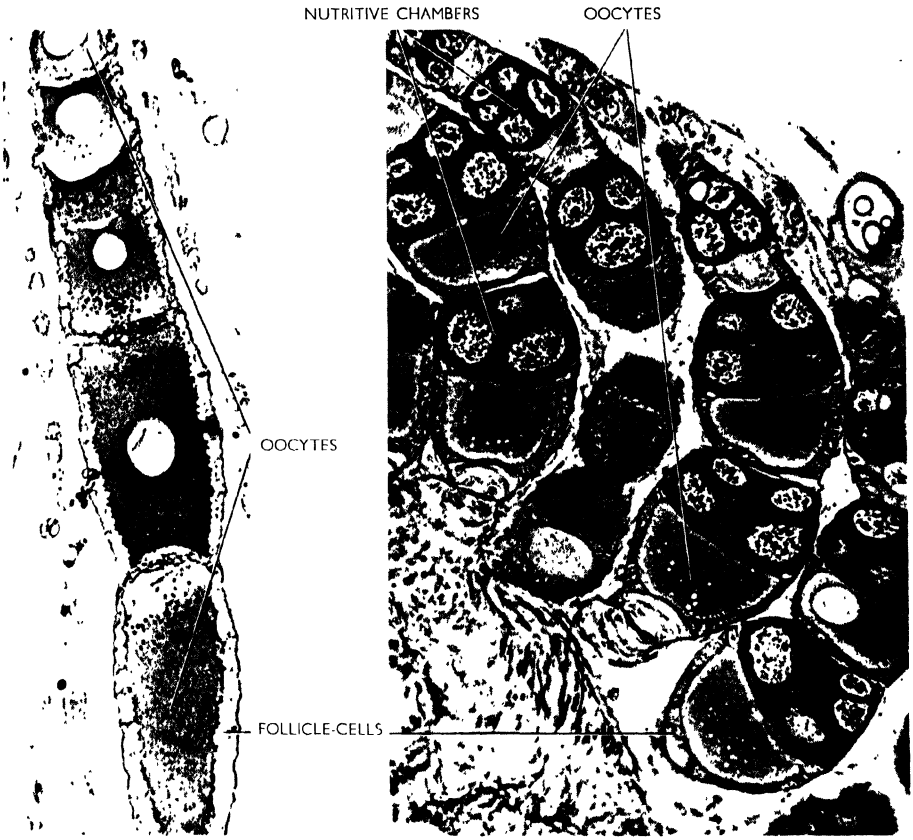


FIG. 29. Photomicrographs. a, middle and posterior part of an ovariole of *Blatta orientalis*. There are no nutritive chambers. Yolk globules are present in the larger oocytes. b, middle and posterior parts of ovarioles of *Stenophylax stellatus*. Nutritive chambers are present. × 100.

Comparatively little work, using modern methods of technique, has been carried out on the mammalian egg, but in the animals examined the Golgi bodies and the mitochondria behave according to the same general plan, and the changes in their distribution are correlated with the stages of development of the oocyte. Even in the eggs of mammals which contain considerable quantities of yolk, such as those of the guinea-pig, there is no evidence that the Golgi elements or the mitochondria take a direct part in yolk-formation. A knowledge of the activities of the Golgi material in the eggs of certain invertebrates, and in gland-cells, makes it reasonable to suppose that it plays some part in the separation from the cytoplasm of the material from which the yolk is formed.

In the young oocytes of certain invertebrate animals the Golgi material is made up of individual elements lying at one side of the nucleus. Later, the elements are distributed through the cell, and in many cases it has been shown that the yolk spheres make their appearance in close association with them. There is, however, disagreement as to the nature of the yolk which arises under the influence of the Golgi material. Certain workers state that the Golgi bodies are concerned with the elaboration of protein yolk, while others, working on different animals, maintain that fatty yolk is formed in association with the Golgi material. Nath (1933) and others believe that Golgi bodies are transformed into fatty yolk globules (p. 139).

It has been suggested that the Golgi bodies, and perhaps the mitochondria, take part in yolk-formation by assisting in the separation of material from the cytoplasm. This material is derived from the cytoplasm and may be added to by nuclear extrusions, substances originating within the follicle-cells, and in certain animals by material derived from nutritive cells associated with the oocytes. The infiltration of Golgi substance from the follicle-cells to the oocyte has been described by several workers (Bhattacharya, 1931; Lal, 1933; and Singh, 1938).

Nutritive, or nurse-cells, are often associated with the oocytes of invertebrates and are a conspicuous feature of the ovary of many arthropods. In insects the ovaries are composed of egg tubes, or *ovarioles*, which unite to open into the oviduct. The apex of a young ovariole is occupied by undifferentiated cells which give rise to oocytes, follicle-cells and in certain cases to nurse-cells. The latter may form *nutritive chambers* situated in relation to each of the oocytes, so that an ovariole consists of oocytes alternating with nutritive chambers (fig. 29, b). In some cases the follicle-wall between the chambers and the older oocytes breaks down and material from the degenerating nutritive cells flows into the oocytes. In certain insects a single group of nurse-cells is connected by protoplasmic bridges with the oocytes, while in others the ovarioles do not contain nutritive cells (fig. 29, a).

THE STRUCTURE OF THE SPERM

The sperms of different animals show wide variations of size and form, and there are often considerable differences between those of related species (fig. 30). The majority possess a *flagellum*, but *non-flagellate* sperms occur

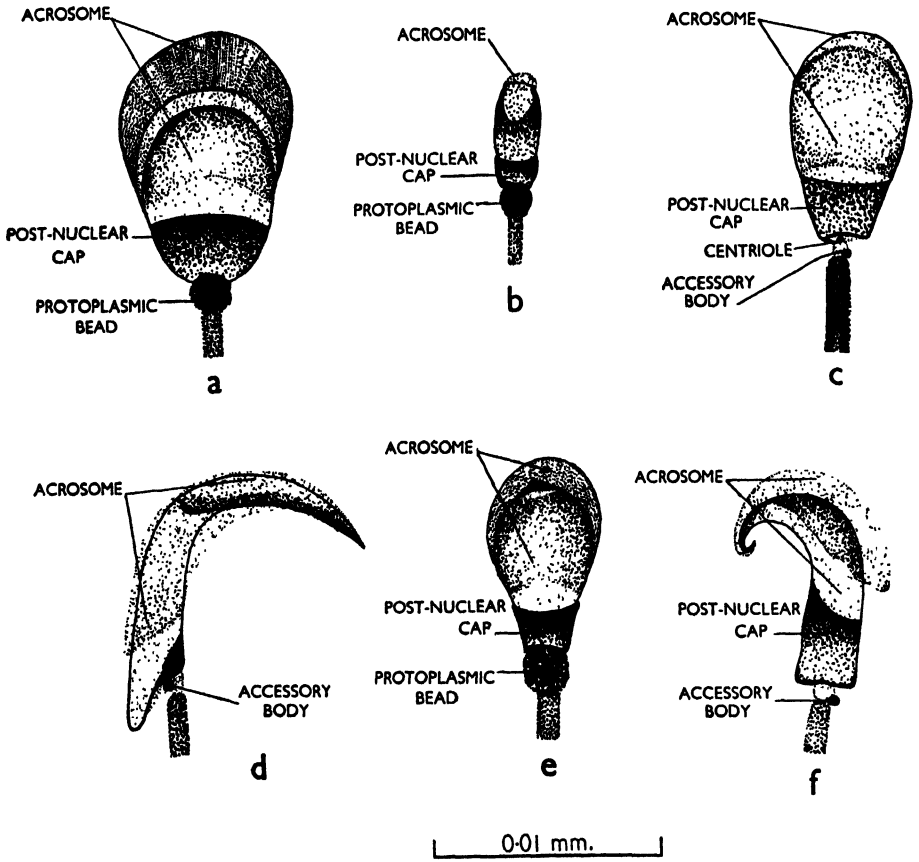


FIG. 30.—The head and anterior part of the middle-piece of the sperms of several mammals. Original drawings. a, guinea-pig. b, cat. c, pig. d, rat. e, rabbit. f, golden hamster. The sperm of the pig, rat, and the golden hamster are drawn from smears of the epididymis ; the protoplasmic bead has been eliminated. The other figures are drawn from sections of the testis.

in nematodes and in certain arthropods. Non-flagellate sperms often undergo slow movements which are either amoeboid or are due to the possession of spine-like processes. Typically the flagellate sperm is divided into the following regions—head, neck, middle-piece and flagellum. The middle-piece varies in different animals, and it is probable that the term has been applied to different structures. Its posterior limit is often marked by the presence of a ring-centriole. Atypical flagellate sperms have been

described in many invertebrates, and the sperms of certain Turbellaria are biflagellate. Dimorphic sperms are produced in the testes of certain

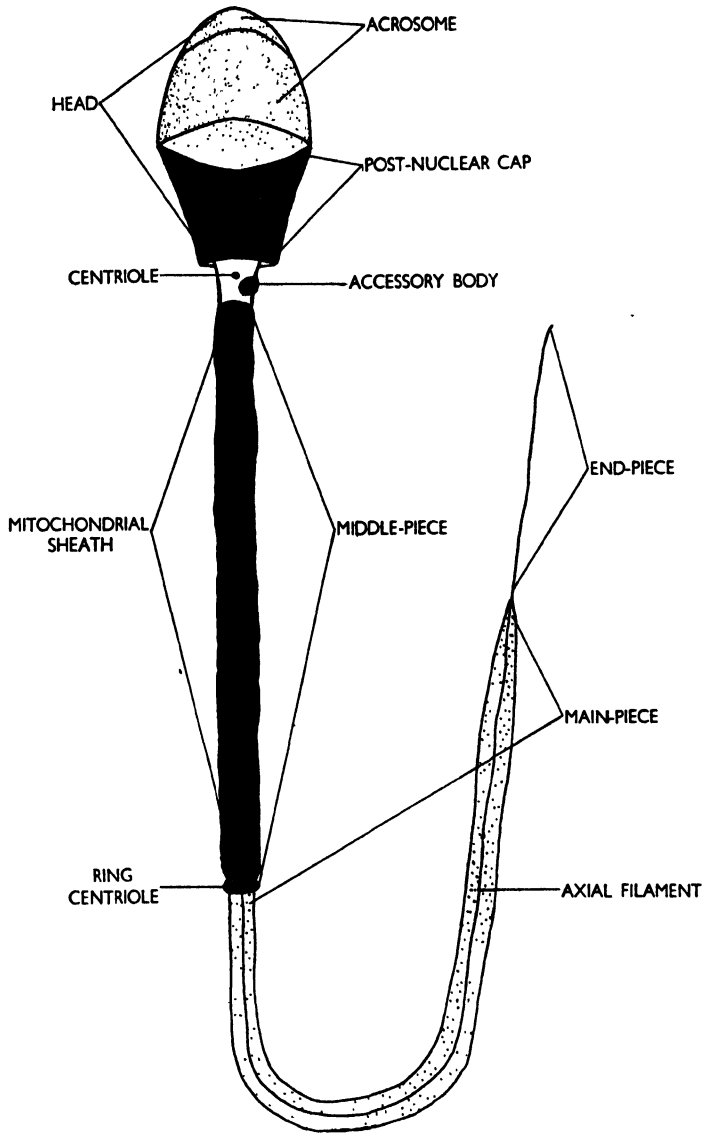


FIG. 31.—Generalized diagram of the mammalian sperm.

invertebrates, where, in addition to the normal, or *eupyrene*, sperms, *oligopyrene* sperms with less than the normal number of chromosomes, and *apyrene*, or non-nucleated, sperms may be present. These arise as abnormalities and ultimately degenerate. Spermatozoa which are larger than normal are sometimes present and may originate through irregularities

of the spermatocyte divisions. In insects, sperms which vary in size are often produced in the different lobes of the testes; it is not known if they are all functional.

The following is a generalized account of the structure of the mammalian spermatozoon (fig. 31). The *head* varies in size and form in different animals and is made up of the following parts: The *nucleus* constitutes the main part of the head. With basic nuclear stains it usually appears as a homogeneous deeply stained mass, but in some cases the cortical part is more deeply stained than the central region. The anterior part of the nucleus is covered by a structure known as the *acrosome* which assumes various forms in different animals. In the guinea-pig it is a blunt cap-like structure divided into an inner and an outer zone, while in some rodents it is curved and hook-like (fig. 30). The whole head is believed to be enclosed in a thin protoplasmic sheath. The posterior part of the nucleus is covered by the *post-nuclear cap*.

The *neck* connects the head with the middle-piece, and is bounded by a continuation of the protoplasmic sheath of the head. The *axial filament* begins in the neck and is continued into the middle-piece and flagellum. A centriole serves as a *basal granule* for the axial filament. There is evidence that the axial filament is composed of fine fibrillae and that it is a contractile structure responsible for the lashing movement of the tail. There is also evidence that Golgi material is present in the neck.

The middle-piece.—The protoplasmic sheath of the neck is continued over the middle-piece. The axial filament is enclosed in a sheath which is formed by some of the mitochondria of the spermatid. The sheath is called the *mitochondrial sheath* and often appears to have a spiral structure. The posterior limit of the middle-piece is marked by the presence of the *ring-centriole*.

The *flagellum* is made up of two parts—the *main-piece* and the *end-piece*. The main-piece is the longest part of the flagellum and is composed of the axial filament surrounded by a protoplasmic sheath. The end-piece consists of the terminal part of the axial filament and is not enclosed by a protoplasmic sheath.

SPERMATOGENESIS

Gatenby and Woodger (1921) and Gatenby and Wigoder (1929) were the first cytologists to give a comprehensive account of the stages of spermatogenesis of a mammal based on the examination of material treated by methods which demonstrate the Golgi substance as well as other important components of the male germ-cell. The following account of spermatogenesis and spermateleosis in the guinea-pig is based on their observations. Since the publication of these two papers, other work has been carried out on mammalian spermatogenesis, and this will be discussed

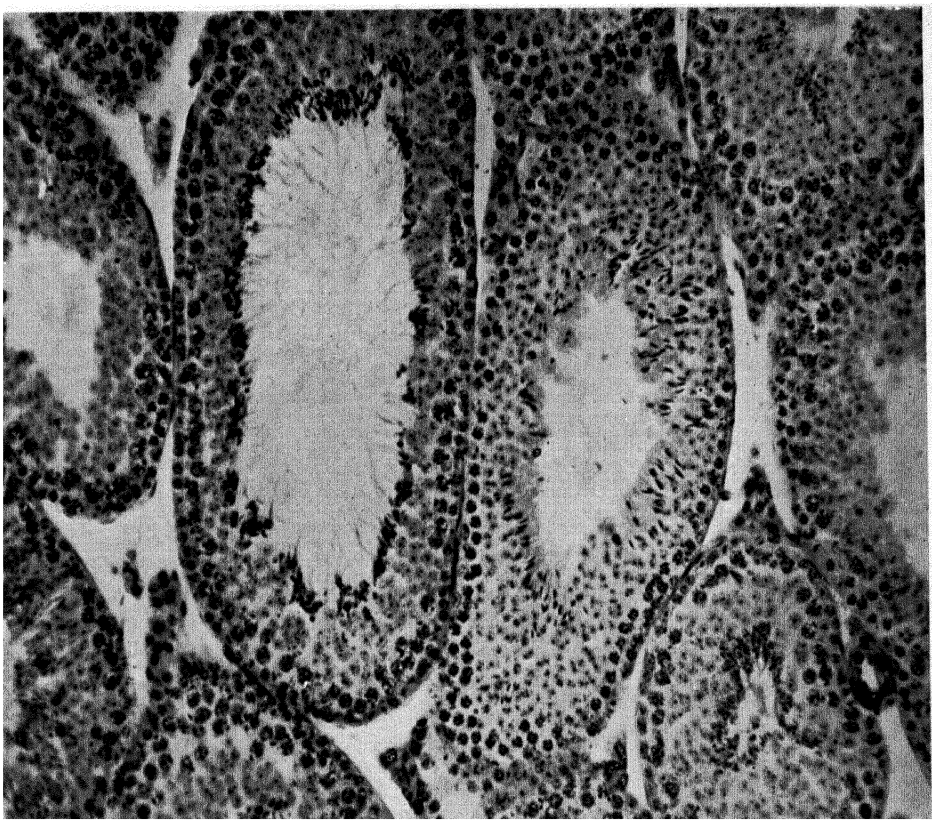


FIG. 32.—Photomicrograph of a transverse section of the testis of the rat. Spermatogonia are present on the outside of the seminiferous tubules immediately beneath the germinal epithelium. The nuclei of most of the spermatocytes are deeply stained and are in the prophase. Numerous spermatids are present between the spermatocytes and the spermatozoa. The tails of the spermatozoa project into the lumen. × 420.

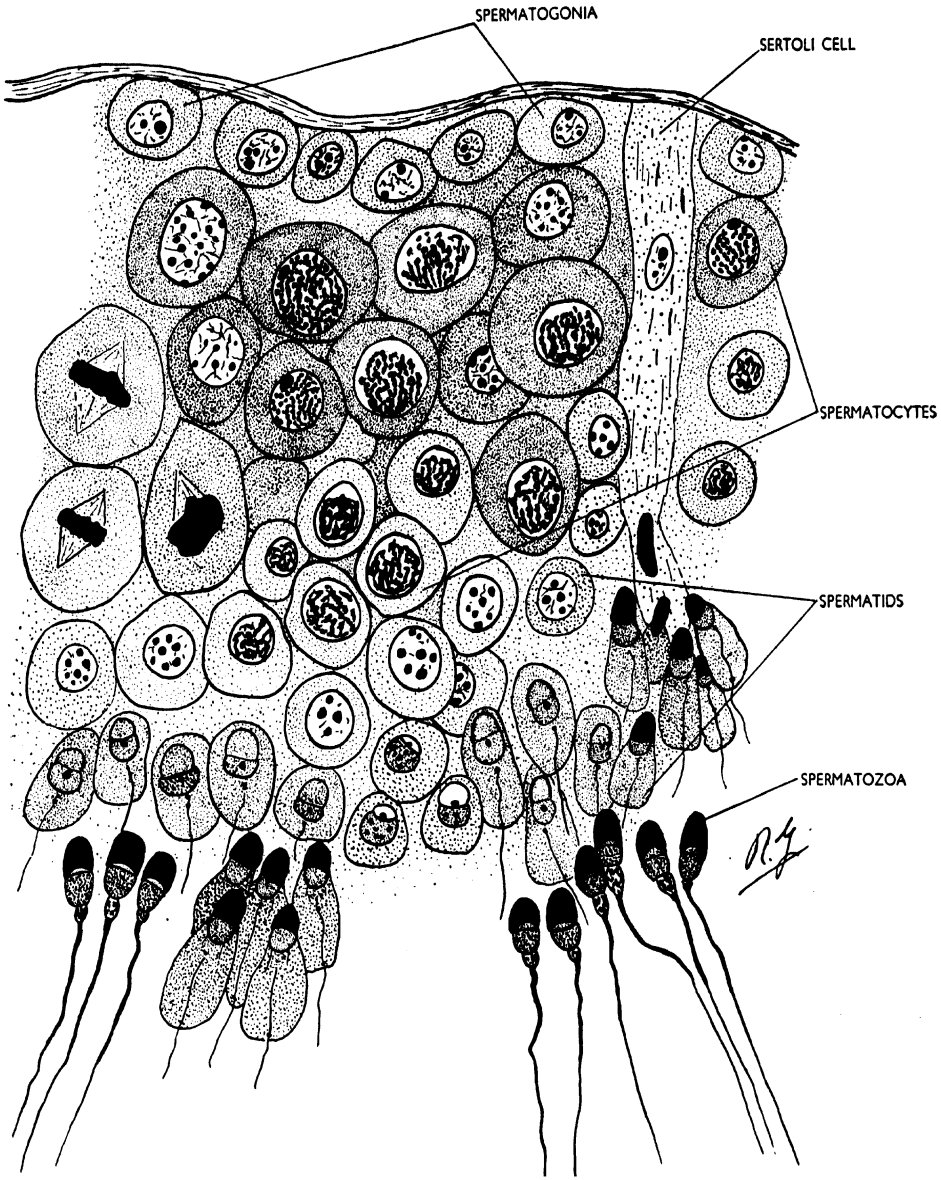


FIG. 33.—Drawing of a small part of a transverse section of a seminiferous tubule of a mammal, to show the stages of spermatogenesis. Semi-diagrammatic.

in so far as it throws further light on the history and structure of the male germ-cells (figs. 32 and 33).

THE SPERMATOCYTES.—The mitochondria of the primary and secondary spermatocytes of the guinea-pig are scattered through the cell; they remain dispersed during the two divisions and are transmitted to the spermatids (fig. 34, a and b). The Golgi material, in the form of curved plates and rods, surrounds the archoplasm. Prior to cell division the localized mass of Golgi material breaks up, the elements become grouped round each pole of the spindle and are distributed in approximately equal numbers to the spermatids (fig. 34, a and b). Argentophil granules are present near the Golgi material of the late spermatocyte; it is probable that they form the *post-nuclear cap* of the spermatid. Small granules, called the *proacrosomic granules*, make their appearance in the archoplasm, and during division are scattered round the spindle.

The Golgi material of the young spermatid surrounds the archoplasm. The proacrosomic granules are within the archoplasm, and each granule is later surrounded by a vacuole—the *archoplasmic vacuole*. The mitochondria are granular and are distributed through the cell. The post-nuclear granules are larger than those of the spermatocyte and are situated at the posterior pole of the nucleus. A centriole is present in the cytoplasm close to the Golgi material. Each spermatid now undergoes a series of changes and is transformed into a spermatozoon; this metamorphosis is known as *spermateleosis*.

SPERMATELEOSIS.—In the young spermatid the proacrosomic granules run together to form two or three larger granules which finally fuse to form a single structure—the *proacrosome*. The latter lies in a vacuole and soon becomes differentiated into an inner and an outer zone (fig. 34, c). Meanwhile, the Golgi material and archoplasm migrate to the opposite pole of the nucleus; the centriole divides into two and the *axial filament* grows out from the *distal centriole*. The mitochondria are still scattered through the cytoplasm.

The proacrosome comes in contact with the anterior pole of the nucleus, and is now known as the *acrosome*. Its two zones rapidly increase in size, and later the Golgi material and archoplasm move towards the posterior region of the cell (fig. 34, d). The spermatid now elongates, the acrosome becomes flattened over the anterior part of the nucleus and assumes its final form. A few elements separate from the mass of Golgi material and later take up a position at the anterior end of the middle-piece. The post-nuclear granules become arranged round the posterior pole of the nucleus and finally fuse to form the *post-nuclear cap*, which appears to be a supporting or uniting structure between the sperm-head and tail. Most of the mitochondria collect round the axial filament and form the *mitochondrial sheath* of the middle-piece (fig. 34, e and f). The two centrioles are now close to the posterior pole of the nucleus; the distal one divides

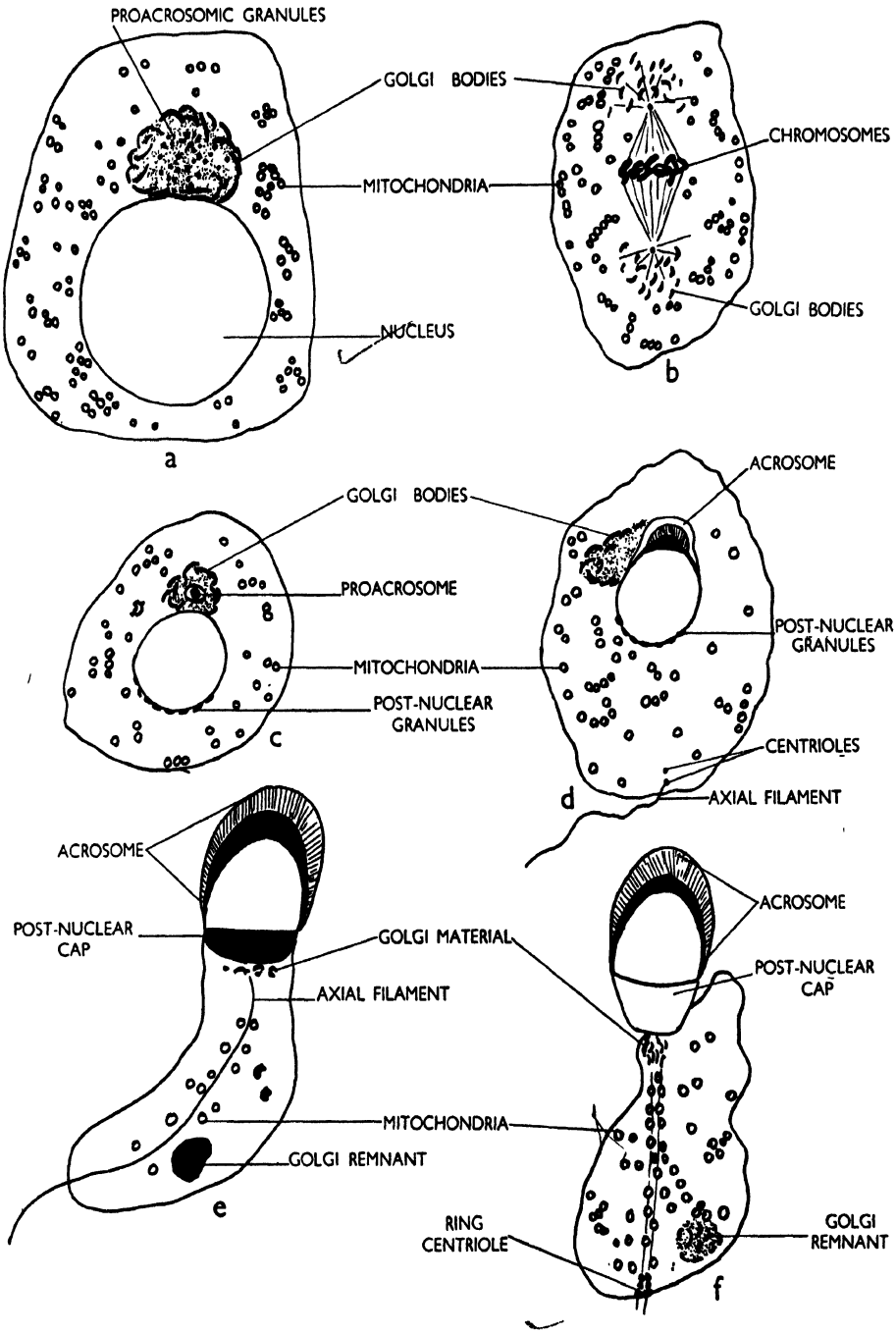


FIG. 34.—Stages of spermatogenesis of guinea-pig. a, primary spermatocyte. b, second spermatocyte ; metaphase. c, young spermatid. d, older spermatid. e and f, stages of metamorphosis of spermatid. a-d and f after Gatenby and Woodger, redrawn and modified. c and d, the post-nuclear granules are included after drawings by Gatenby and Wigoder. e, after Gatenby and Wigoder, redrawn and modified.

and one of the products becomes ring-shaped and moves to the posterior end of the middle-piece. (More recently Gatenby and Beams, 1935, and others, have stated that the distal centriole does not divide, but becomes ring-shaped.) Practically all the cytoplasm is sloughed off; this *residual cytoplasm* contains mitochondria which have not entered into the formation of the middle-piece, and degenerating Golgi elements known as the *Golgi remnant*. When fully formed the sperms pass into the lumen of the seminiferous tubules and are conveyed to the epididymis.

The more recent work of Gresson on the mouse (1942), and of Gresson and Zlotnik (1945) on certain mammals, indicates that the elements which separate from the Golgi material, while the latter is situated in the cytoplasm posterior to the nucleus of the spermatid, enter a *protoplasmic bead* present on the neck of the spermatozoon (fig. 35, g and h). Gresson and Zlotnik claim that bodies originate from the Golgi material of the spermatocyte and spermatid, and that one of these is present in the neck region of the late spermatid and the spermatozoon (fig. 35). They believe that these structures are identical with bodies previously seen, but not studied in detail, by other workers and called by Gatenby and Beams (1935) *accessory bodies*. In the rat the rim of the accessory body of the spermatid is sometimes faintly impregnated with silver nitrate, but in the other mammals studied the whole body impregnates deeply. Gresson and Zlotnik suggest that the accessory body may be comparable to the Golgi pre-substance of Hirsch (p. 130), that the time at which it becomes argentophil is not the same in all animals, and that it is transformed into the Golgi material of the sperm.

Gatenby and Collery (1943) speak of two beads present in the sperm of the dog—an anterior one which corresponds in position with the region believed by Gresson and Zlotnik to contain an accessory body, and a posterior one which is identical with the protoplasmic bead mentioned above. Collery (1943) thinks that the posterior bead is probably acquired while the sperms are within the epididymis, while Gresson and Zlotnik state that their preparations show clearly that it is formed while the sperms are within the testis and that it is lost in the epididymis (fig. 36).

Gresson and Zlotnik believe that the archoplasmic vacuole, as well as the archoplasmic granule, takes part in the formation of the acrosome. They state that the vacuole comes in contact with the nucleus, increases greatly in size, and depresses the anterior region of the nucleus (fig. 35, b and c). After the vacuole has reached its full size the nucleus grows out underneath it (fig. 35, c and d); the vacuole disappears and material which it contains apparently contributes to the formation of the acrosome.

As the proacrosomic granules arise within the archoplasm it is probable that the Golgi material plays some part in their origin, and further, that the substance contained in the vacuole is formed under the influence of the Golgi material. Investigations on the origin of the acrosome of other

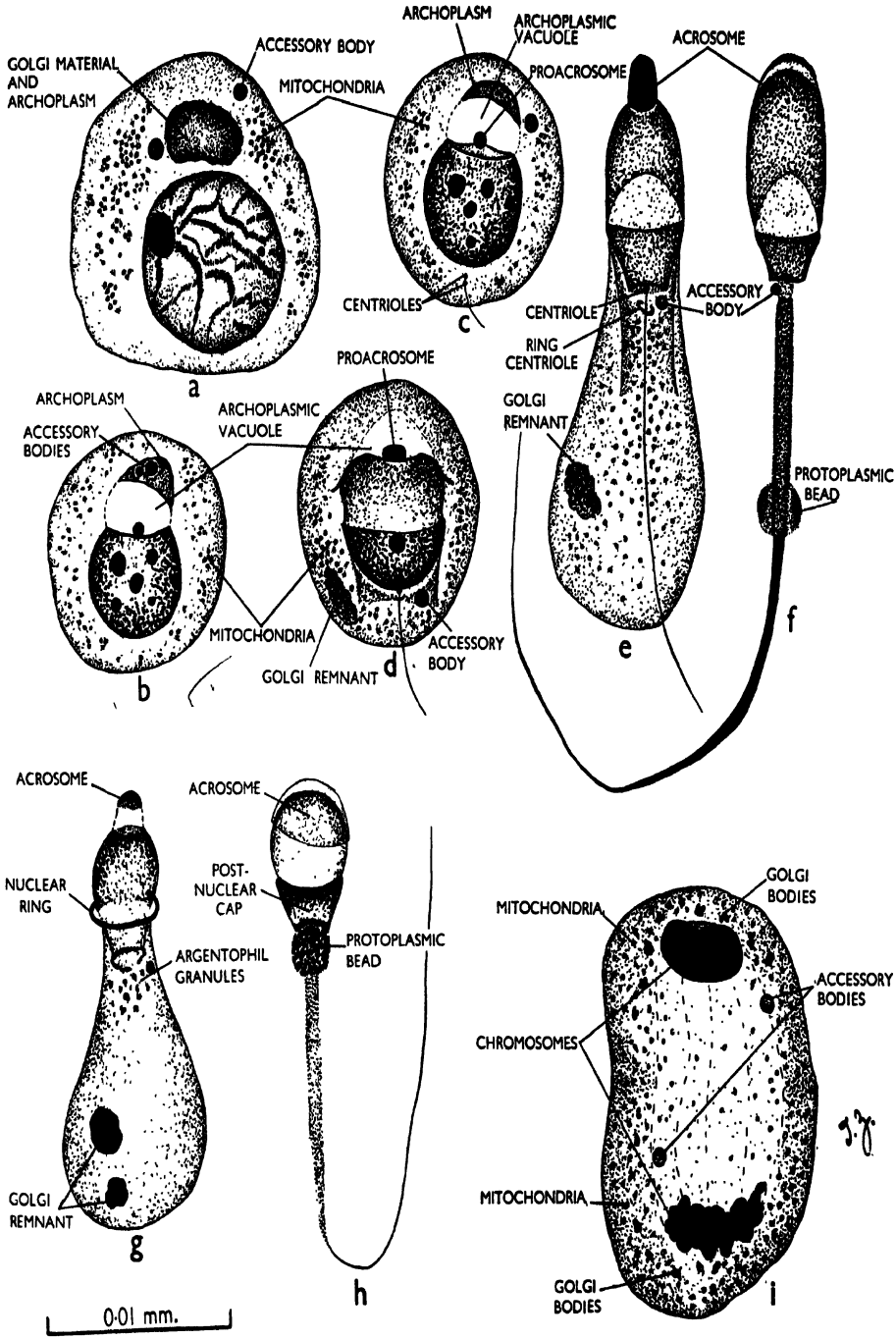


FIG. 35.—Stages of spermatogenesis of mammals. Original drawings. a, primary spermatocyte of sheep. b-d, spermatids of sheep. e, late spermatid of sheep. f, sperm from epididymis of sheep. g, late spermatid of dog. h, sperm from seminiferous tubule of dog. i, primary spermatocyte of sheep ; telophase.

animals support the view that the Golgi substance plays an important part in the process. In the Lepidoptera, and in certain other animals, a *proacrosome bead* is secreted under the influence of Golgi bodies.

A ring-shaped structure encircling the nucleus of the spermatid and the head of the sperm of the dog and the cat was first described by Zlotnik (1943). The *nuclear-ring* is formed at the edge of the depression caused by the pressure of the archoplasmic vacuole on the nucleus; later, it stains deeply and forms a conspicuous structure (fig. 35, g). The same observer claims that a second ring is present at the posterior pole of the nucleus Nuclear-rings have since been observed in other mammals.

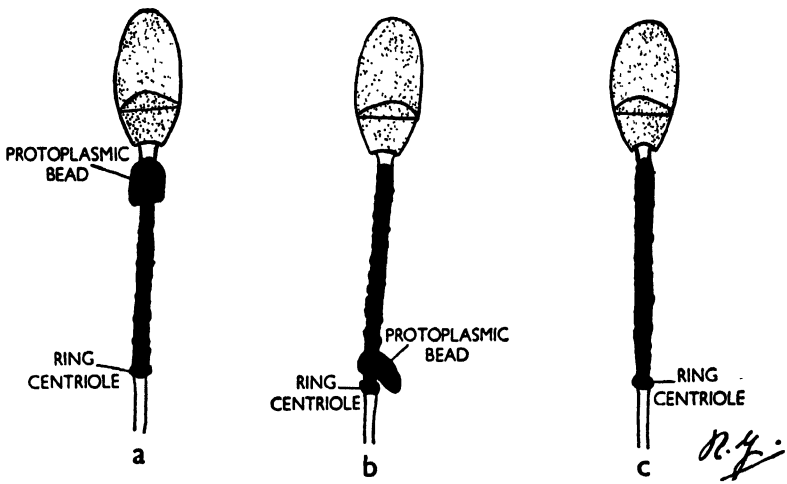


FIG. 36.—Sperm from the epididymis of the sheep. Original drawings. a, sperm which has recently entered the epididymis and possesses a protoplasmic bead immediately posterior to the neck. b, the protoplasmic bead has moved down to the posterior part of the middle-piece. c, mature sperm after the elimination of the protoplasmic bead.

This short account of the development of the mammalian sperm shows that it is a complex structure, that several components of the spermatid enter into its composition, and that there is a risk of confusion between the various argentophil bodies present in the late spermatid. Granules have been observed in the spermatids of invertebrates, and Gatenby (1941) in a short review has discussed these structures. It is desirable that further work be carried out on the cytoplasmic components of the male germ-cells; it has been established, however, that the mitochondria and Golgi elements of many other animals behave in essentially the same way as those of the mammalian spermatid.

To summarize. Many details of the structural transformations which take place in mammalian spermateleosis have been observed in recent years. Animals such as the guinea-pig, the rat, the rabbit, the pig and the sheep, are convenient subjects in which to study the history of the

cytoplasmic components. The sheath which surrounds the axial filament of the sperm is mitochondrial in origin. The Golgi material is concerned with the formation of the acrosome. The function of the latter is not known with certainty; it was formerly thought to aid the sperm in becoming attached to, or in penetrating, the egg, and it has been suggested that it contains some substance which takes part in the activation of the ovum. Recent work indicates that the distal centriole does not divide, but becomes ring-shaped and moves down to the posterior end of the middle-piece. A post-nuclear cap is present in most of the mammalian sperms examined with suitable methods of technique; in some animals it is deeply impregnated with silver nitrate while in others it is lightly impregnated. An accessory body is included in the neck region and probably forms the Golgi material of the ripe sperm. The presence of a nuclear-ring in the late spermatid and in the sperm has been established. A protoplasmic bead containing Golgi elements is present on sperms within the testis, but is lost in the epididymis. While there have been considerable advances in our knowledge of the structure of the male germ-cells, our understanding of the functions of the cytoplasmic components is still far from complete.

CHAPTER VIII

FERTILIZATION, PARTHENOGENESIS, AND THE ORIGIN OF THE PRIMITIVE GERM-CELLS OF SOME ANIMALS

FERTILIZATION is the union of the spermatozoon and ovum to form the fertilized egg or *zygote*; the union of the gametes is often spoken of as *syngamy*, and the union of the gamete-nuclei as *karyogamy*. Fertilization comprises a number of physiological and morphological phenomena which take place in the period between the entry of the sperm and the fusion of the gamete-nuclei. The process results in the activation of the egg, leads to cell division, the formation of a new individual, and to the combination of a maternal and a paternal group of chromosomes in the nucleus of the zygote.

The cells which unite in fertilization are sexually differentiated. The female cell, or ovum, contains a relatively large amount of cytoplasm, and frequently is provided with considerable quantities of nutritive material. The male cell, or spermatozoon, contains little cytoplasm, and therefore its chief quantitative contribution to the zygote is nuclear material. It is an active cell, usually possessed of considerable power of movement, enabling it to swim to the egg by the lashing of its flagellum or by other means. The ovum is a relatively passive cell and does not appear to take an active part in the initial stages of fertilization, except that in some animals a cone, called the *fertilization* or *attraction cone*, is formed at the point at which the spermatozoon enters. The cone is said to engulf the sperm and facilitate its entry. In many animals one sperm only penetrates the ovum and induces physiological changes in the egg which prevent the entry of other spermatozoa; the nature of these changes is not clearly understood. *Polyspermy* occurs in most insects and in several animals with large eggs; the nucleus of one sperm fuses with the egg-nucleus and the other sperms degenerate, but in some cases their nuclei may first become vesicular.

Immediately after the penetration of the sperm the *fertilization membrane* rises off the surface of the egg, and a space, filled with fluid and known as the *peri-vitelline space*, appears between the ovum and the surrounding membranes. In certain mammals some of the cytoplasm of the egg, together with yolk-globules, is passed into the peri-vitelline space; this process is called *deutoplasmolysis*.

There is considerable variation in the relationship between the time of entry of the sperm and the maturation of the ovum. In sea-urchins and some other animals maturation takes place before ovulation, and fertilization is said to be of the *Echinus type*. In some animals the egg-nucleus is in the germinal vesicle stage, or at some stage of the meiotic divisions, at the time of ovulation. In this case the entry of the sperm acts as a stimulus causing the egg to complete its maturation. When the stages of the first meiotic division, with the exception of the early prophase, take place after the entry of the sperm, fertilization is said to be of the *Ascaris type*. In many animals fertilization is intermediate in character between these two types.

ECHINUS TYPE OF FERTILIZATION

In *Echinus* (fig. 37), at the time of ovulation, the egg-nucleus is in the " resting stage " and is known as the *female pronucleus*. The spermatozoon enters the ovum head first, the middle-piece becomes detached from the head and a centriole is liberated from the neck region. The sperm-nucleus rotates and, preceded by the centriole, moves towards the female pronucleus; the sperm-nucleus is now called the *male pronucleus*. Astral rays grow out from the centriole, and at the same time the male and female pronuclei move towards the central region of the egg, come into contact with each other, and fuse while still in the " resting stage ". The sperm centriole divides either immediately before or after the fusion of the pronuclei and provides the centrosomes for the first cleavage division; a spindle is formed, the chromosomes become visible, and the nuclear membrane disappears. The chromosomes become arranged at the equator of the spindle and pass through the usual anaphase and telophase changes. The cell divides to form the first two *blastomeres*, and by repeated divisions gives rise to a new individual. The *Echinus* type of fertilization is not common.

ASCARIS TYPE OF FERTILIZATION

In *Ascaris* (fig. 38) the nucleus is in the germinal vesicle stage when the egg enters the oviduct; it is large and lies between the central region of the cell and the periphery. After the spermatozoon penetrates the cortical region of the ovum the sperm-centriole is liberated and the head rotates; at the same time the egg-nucleus migrates towards the periphery and enters upon the metaphase of the first meiotic division. The first polar body is formed, and the nucleus immediately divides again to form the female pronucleus and the second polar body. The female pronucleus moves towards the central region of the egg; meanwhile the male pronucleus has increased in size and assumed a vesicular structure. The sperm-centriole divides and a small spindle makes its appearance. The

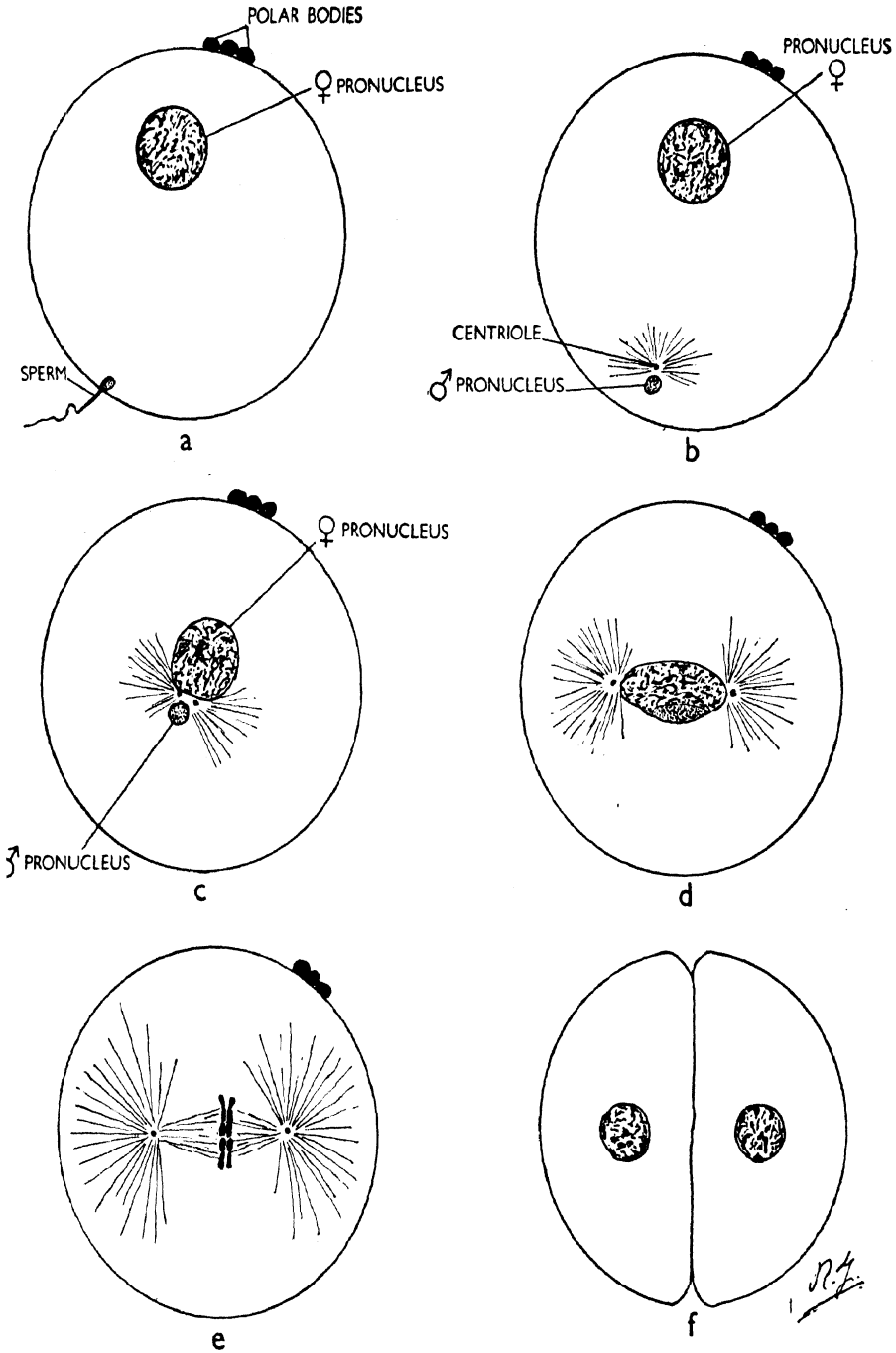


FIG. 37.—*Echinus* type of fertilization. Diagrammatic. a, entry of sperm. b, to show sperm centriole. c, the male pronucleus has increased slightly in size ; the centriole has divided. d, fusion of pronuclei. e, metaphase of first cleavage division. f, the first two blastomeres.

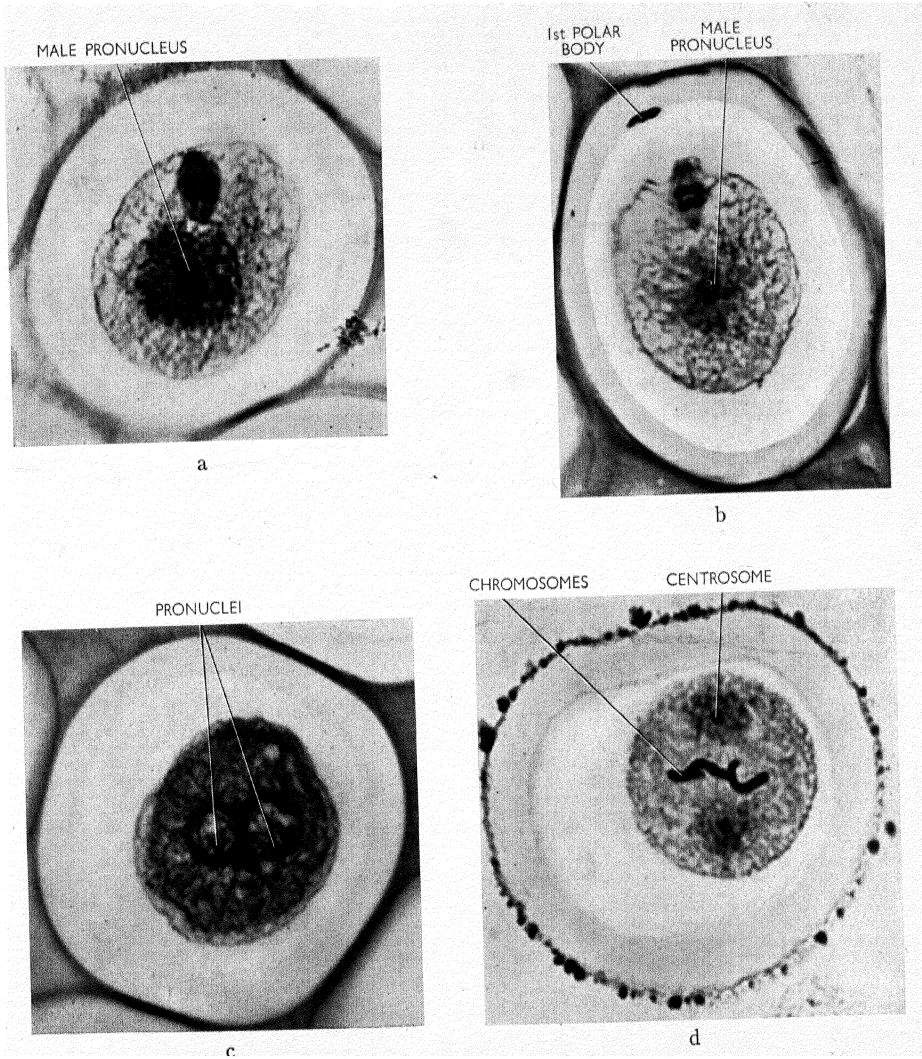


FIG. 38.—Photomicrographs of stages of fertilization of *Ascaris equorum*. a, formation of the first polar body. b, formation of the second polar body. c, male and female pronuclei in contact. d, metaphase of first cleavage division. ×780.

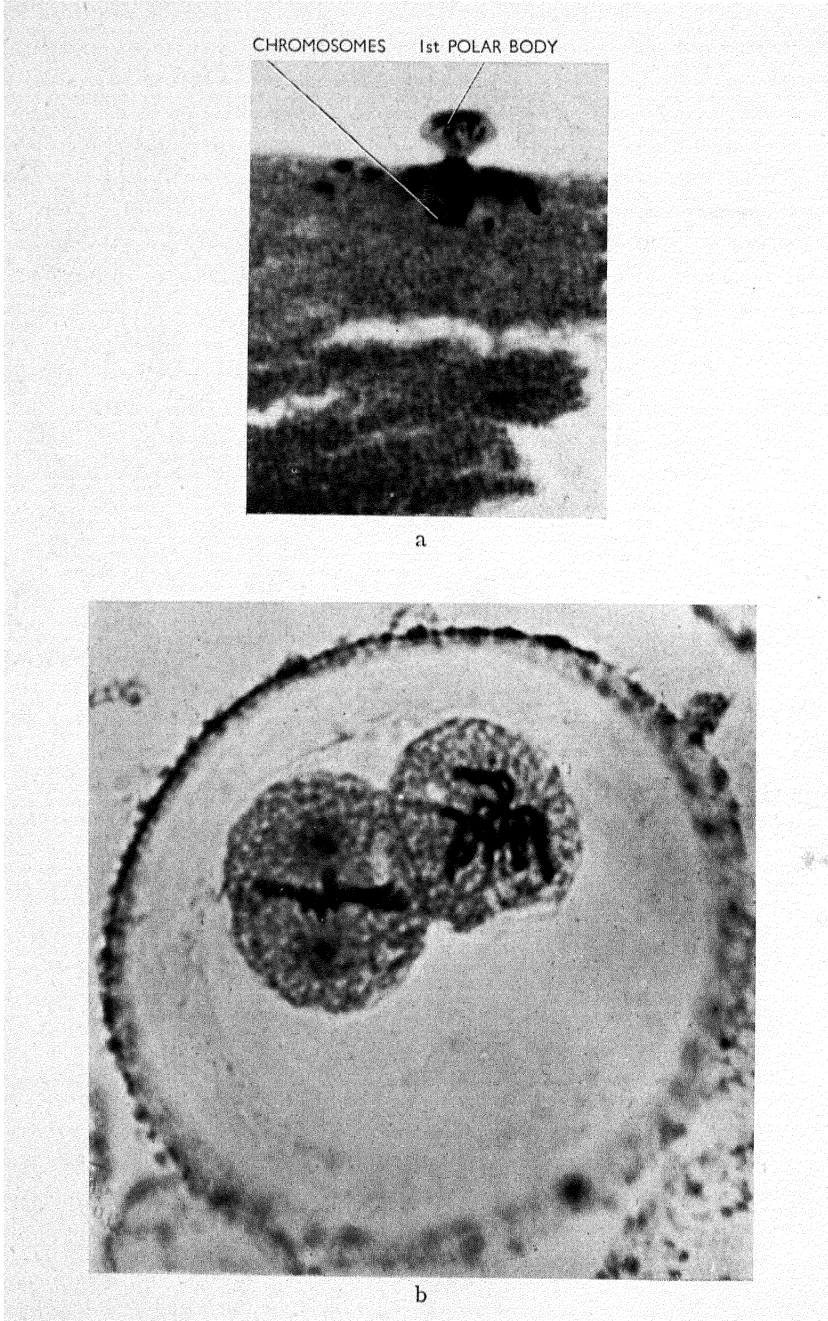


FIG. 39.—Photomicrographs. a, egg of whitefish ; formation of the first polar body. At the second maturation division the group of chromosomes within the egg will divide to form the second polar body and the female pronucleus. b, an early cleavage stage of *Ascaris equorum*. One of the cells is in the late prophase and the other is in the metaphase. × 1200.

male pronucleus, preceded by the centrioles and spindle, moves towards the female pronucleus, and, as it migrates into the deeper regions of the egg, increases still further in size and becomes approximately equal to the female pronucleus. Before the pronuclei come in contact with each other, the chromosomes of each become visible and go through prophase changes. Consequently, the pronuclei fuse while in mitotic prophase and the chromosomes immediately take up their metaphase positions. The metaphase is followed by the anaphase and telophase and the fertilized egg divides to give the first two blastomeres.

In the *Ascaris* type of fertilization the male pronucleus may become as large as the female pronucleus, and the paternal and maternal chromosomes are visible before nuclear fusion. As in *Echinus*, the sperm-centriole gives rise to the division centres for the first cleavage division; it divides, however, while the male pronucleus is still in the peripheral region of the egg.

FERTILIZATION IN SOME OTHER ANIMALS

Intermediate conditions have been observed in many animals. For example, in *Cerebratulus* the sperm penetrates the egg during the metaphase of the first meiotic division, and in the mouse the egg-nucleus remains at the anaphase of the second maturation division until the entry of the spermatozoon. In the intermediate types the sperm-nucleus may reach a size approximately equal to that of the female pronucleus.

In some animals the maternal and paternal chromosomes form separate groups during the early stages of embryology, and in certain cases—for example, *Cyclops*—double spindles and nuclei are recognizable in the early cleavage stages. When the chromosomes remain in two separate groups the condition is known as *gonomery*.

THE CYTOPLASMIC COMPONENTS DURING MATURATION AND FERTILIZATION

The behaviour of the mitochondria has been followed through the stages of maturation and fertilization in certain animals of widely separated groups, and the Golgi material has been studied in the fertilized egg of the rabbit and the mouse. The sperm middle-piece has been observed in the ova of many animals, and in some cases the granular structure of the mitochondrial sheath was visible. The mouse, however, appears to be the only mammal in which the history of the Golgi material of both the egg and the sperm has been investigated in detail during fertilization. Lams and Doorme (1908) gave a brief account of the behaviour of the mitochondria in the fertilized egg of the mouse. They observed the sperm-head and middle-piece in the cytoplasm of the egg, but did not

follow the history of the middle-piece beyond the stage of the pronuclei. Gresson (1941) traced the history of the Golgi material and mitochondria as far as the two-cell stage, and claimed that both mitochondria and Golgi

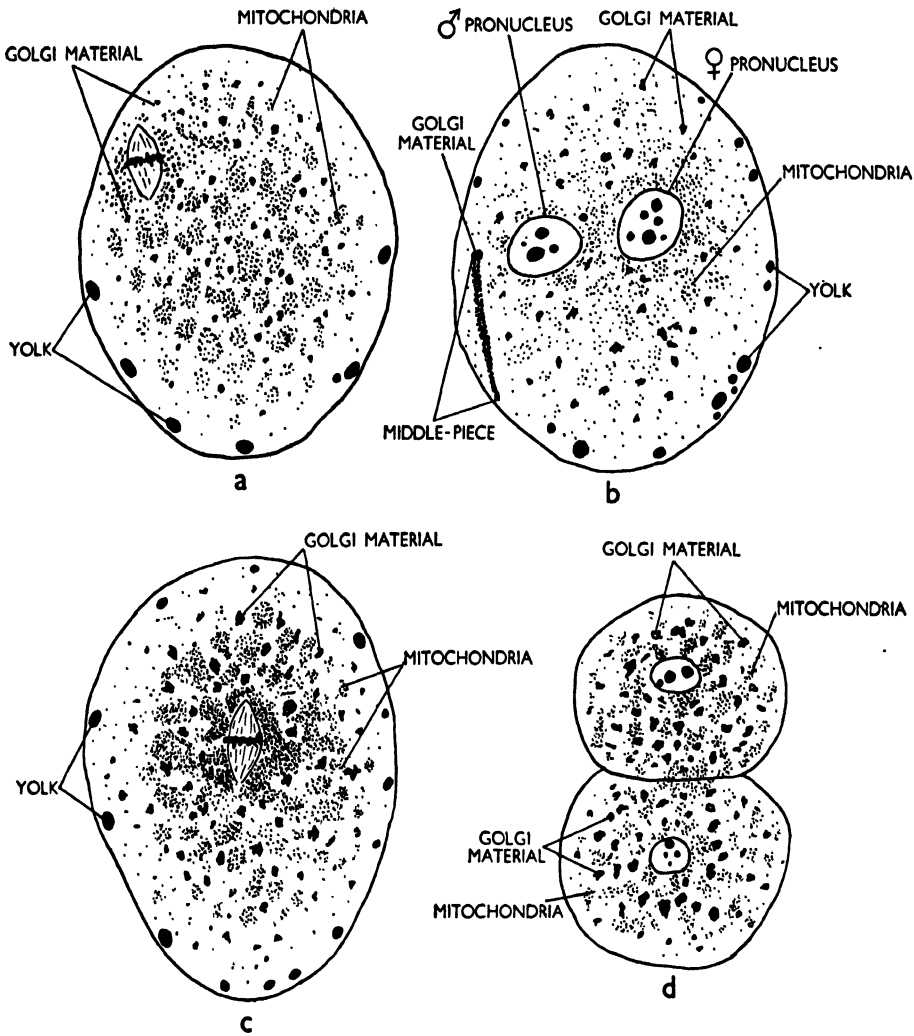


FIG. 40.—The mitochondria and Golgi material during maturation, fertilization, and the first cleavage division of the egg of the mouse. a, first maturation division. b, fertilization. c, first cleavage division. d, the first two blastomeres. After Gresson, redrawn and modified.

elements are introduced by the sperm. The following account is based on the more recent work of Gresson.

THE MATURATION DIVISIONS OF THE EGG OF THE MOUSE.—During the first meiotic division (fig. 40, a) most of the granular mitochondria are

collected into clumps, but a few are scattered singly through the cell. The clumps are most numerous in the central region and in the vicinity of the spindle; a few mitochondria are eliminated in the first polar body. The Golgi elements, in the form of granules and of small irregularly shaped bodies, are fairly evenly distributed throughout the cytoplasm. During the second division the distribution of the Golgi material is closely similar to that of the first division. The mitochondria, however, are more numerous in the half of the egg in which mitosis is taking place. In the later stages the spindle is situated at the periphery and is more or less parallel to the surface of the egg, the mitochondria are not numerous at the spindle poles or between the spindle and the cell membrane, and consequently few are present in the second polar body

FERTILIZATION IN THE MOUSE.—The sperm enters the egg during the early anaphase of the second meiotic division. In some cases the head immediately becomes free of the middle-piece, but in others the two parts remain together until the end of the polar division. The mitochondria of the middle-piece are larger and more deeply stained than those of the ovum (fig. 40, b); they soon separate out from their position around the axial filament and form a broad band. Later, they lose their intensity of staining, spread out through the cytoplasm, decrease in size and become indistinguishable from those of the egg. The Golgi material of the sperm is present, before the distribution of the mitochondria, at the anterior end of the middle-piece. It appears to break up into small elements which rapidly migrate through the cytoplasm and lose their identity among the Golgi bodies of the ovum.

THE FIRST CLEAVAGE DIVISION OF THE EGG OF THE MOUSE.—As the pronuclei approach each other the Golgi elements become larger and more numerous; when the spindle of the first cleavage division is formed they are still scattered through the cytoplasm, but are slightly more numerous in the vicinity of the spindle. Many of the clumps of mitochondria collect around the spindle and run together to form larger masses (fig. 40, c). Both the mitochondria and the Golgi elements, as the result of the positions which they occupy, are distributed in approximately equal numbers to the first two blastomeres (fig. 40, d). The Golgi material and mitochondria of the sperm are scattered amongst those of the egg, and at this stage cannot be distinguished from the latter.

THE MIDDLE-PIECE IN THE EGGS OF MAMMALS.—The sperm middle-piece has been observed in the eggs of mammals other than the mouse, but few attempts have been made to follow its history, and conclusions regarding its fate are conflicting. Van der Stricht (1902) stated that the sperm-tail of the bat passes into one of the blastomeres of the two-cell stage; a similar conclusion was reached by Lams (1913) regarding the sperm-tail of the guinea-pig, and Levi (1915) recorded the presence of the tail in one cell of a three-cell stage of the bat. Lams stated that the

cytoplasm of the cell containing the sperm-tail is male and female in origin, and that of the other blastomere female in constitution. In a later paper Van der Stricht (1923) suggested that the embryo is derived from the cell containing the middle-piece, and the trophoblast from the other cell. Kremer (1924) failed to find a sperm-tail in the egg of the mouse and concluded that it disintegrates shortly after entering the cytoplasm. Nihoul (1926) studied the Golgi material and mitochondria of the fertilized egg of the rabbit, and was not convinced that the sperm-tail always penetrates the egg. According to Gresson (1941) the mitochondria and Golgi material of the middle-piece of the sperm of the mouse are distributed in approximately equal amounts to the first two blastomeres.

The study of the behaviour of the cytoplasmic components of the sperm during fertilization is of interest from the point of view of the contribution of the male to the cytoplasm of the zygote. Brambell (1930) pointed out that, " virtually nothing is known of the fate in vertebrates of the sperm mitochondria and Golgi bodies contained in the middle-piece. Yet these various bodies constitute the cytoplasm received from the male parent and are equivalent, from the point of view of cytoplasmic inheritance, to the entire cytoplasm of the egg. It is highly desirable that these structures should be followed, by means of modern technique, through the stages of fertilization and segmentation." That the cytoplasmic components of the mammalian sperm, other than the centriole, play an important part in fertilization is not supported by the earlier investigations cited above. The work, however, was mainly concerned with other aspects of fertilization. In the more recent investigation on the egg of the mouse there is strong evidence that the middle-piece disappears before the first cleavage division, and that the sperm-mitochondria and Golgi elements are distributed through the cytoplasm of the egg where they multiply and are passed on to the first two blastomeres.

THE MIDDLE-PIECE IN THE EGGS OF INVERTEBRATES.—Convincing evidence that the sperm-mitochondria are carried into the egg was produced by the work of Meves on certain invertebrates. In *Ascaris* (1911) and in *Filaria* (1916, a) the mitochondria spread out from the middle-piece, become distributed through the cytoplasm, break up and are transmitted to the first two blastomeres. Meves (1914) traced the middle-piece to one of the blastomeres of the thirty-two-cell stage of *Echinus*, and, in order to explain its presence in one cell only, suggested that the young animal is derived wholly, or mainly, from the blastomere containing the middle-piece, and that the larval structures are formed from cells containing egg-mitochondria only. He observed sperm-mitochondria in the eggs of *Mytilus* (1916, b) but was unable to determine their fate. Meves believed that conjugation takes place between the paternal and maternal mitochondria, but there is no evidence in support of this view.

Held (1917) and Collier (1936) observed sperm-mitochondria in the fertilized ovum of *Ascaris*, and Held claimed that, due to differences in staining properties, the paternal mitochondria can be distinguished even after they have become as small as those of the egg.

In view of the evidence that the middle-piece contributes mitochondria to the fertilized egg, and as it has been claimed that in some animals the middle-piece is segregated into one blastomere, while in others the sperm-mitochondria are scattered through the cytoplasm before the first cleavage division, it is desirable that further work be carried out, using methods which will preserve the Golgi elements as well as those which demonstrate the mitochondria. In the opinion of the writer the problems which await solution in this field of research have been for too long neglected.

PARTHENOGENESIS

The development of an egg into an embryo without karyogamy is called *parthenogenesis*. Parthenogenesis occurs in nature in rotifers, lower Crustacea, insects and in some other invertebrates, and, it is claimed, has been induced experimentally by treating the eggs of certain animals, including mammals, with mechanical and chemical agents. In the life-history of some animals parthenogenesis alternates with sexual reproduction and in others males are very rare or are unknown. In these cases only one polar body is formed and, with the exception of male-producing eggs, there is no reduction of chromosome number. This type of parthenogenesis is called *diploid parthenogenesis*. In certain insects and arachnids fertilized ova give rise to females and unfertilized eggs develop into males by *haploid parthenogenesis*. In rotifers both diploid and haploid parthenogenesis occurs.

In aphids a sexual generation appears in the late summer, and the females produce eggs which are fertilized and hatch in the following spring giving females only. The females which develop from the fertilized ova reproduce by diploid parthenogenesis and give rise to several generations of parthenogenetic females. In the late summer the last parthenogenetic generation gives origin to the sexual generation. The female-producing eggs contain the diploid number of chromosomes, but in the male-producing eggs one or two chromosomes lag behind on the spindle during the anaphase of the maturation division and are not included in the nucleus; consequently the male has fewer chromosomes than the female. In the male-producing ova, therefore, reduction takes place but involves only one or two of the bivalents. The oocytes of the sexual generation undergo reduction to give ripe eggs with the haploid number of chromosomes, but two kinds of sperms are formed—one male-producing as regards its chromosome constitution, and one female-producing. The male-producing sperms, however, degenerate so that all the eggs are

fertilized by female-producing sperms and give females in the following spring which reproduce parthenogenetically.

Haploid parthenogenesis occurs in the Hymenoptera. The nuclei of the female contain the diploid number of chromosomes and those of the male the haploid number. The behaviour of the chromosomes has been followed in the bee and in several other members of the order, and although slight variations exist, both oogenesis and spermatogenesis are essentially similar in all the animals investigated. The egg-nucleus undergoes two maturation divisions and the female pro-nucleus contains the haploid number of chromosomes. The nucleus of the primary spermatocyte, which contains the haploid number of chromosomes, passes through certain prophase changes, but nuclear division does not take place. A small cytoplasmic body separates from the cell (fig. 41, a-c); the latter then enters upon the prophase of mitosis and divides. In some cases division results in one large spermatid and functional sperm, and one small nucleated cell which degenerates (fig. 41, d); in other cases division is equal and gives two spermatids which develop into normal sperms. There is no reduction division and the sperms contain the same number of chromosomes as are present in the spermatogonia. Consequently, the fertilized egg contains the diploid number of chromosomes and develops into a female, and the unfertilized egg develops into a male with the haploid number of chromosomes.

In some animals, if fertilization does not take place, one of the polar bodies fuses with the egg-nucleus and the diploid number is restored.

It is probable that parthenogenetic development has arisen through modifications of the sexual method. A considerable amount of work has been carried out on artificial parthenogenesis and embryos so produced have developed up to a certain stage, but only in a few cases have they undergone metamorphosis or reached sexual maturity. When the artificially activated egg has the haploid number of chromosomes, the diploid condition is frequently restored at some stage of development. Parthenogenesis presents a number of cytological problems, some of which are discussed by Whiting (1945).

THE ORIGIN OF THE PRIMITIVE GERM-CELLS OF SOME ANIMALS

The early cleavage divisions result in an embryo consisting of a large number of cells. By further cell multiplication, differentiation and integration, the tissues of the adult organism are built up. In many animals the cells which give rise to the germ-cells can be identified at an early stage of development; in some cases they are differentiated from the somatic cells during the early cleavage stages, and in certain insects cytoplasm, which is later included in the primitive germ-cells, is visible in

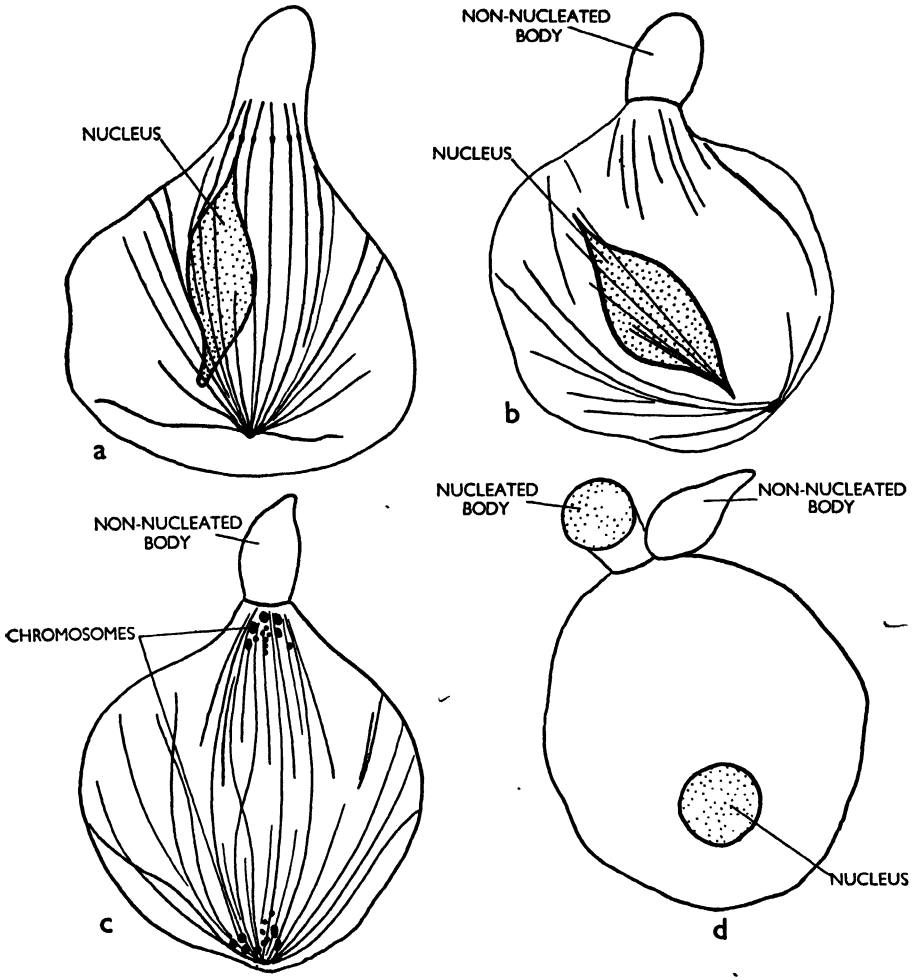
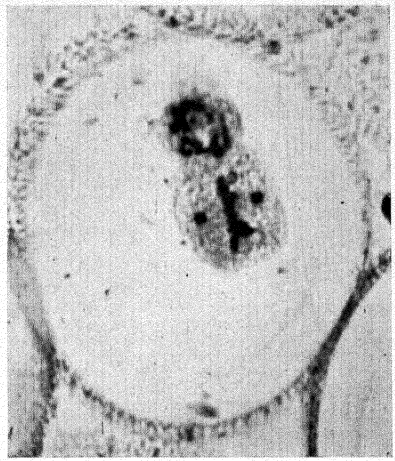


FIG. 41.—Spermatocytes of *Apis mellifica*. a-b, the first or abortive division which results in the formation of a non-nucleated body. c-d, the second maturation division which gives a small nucleated body and one spermatid. After Meves, redrawn and modified.

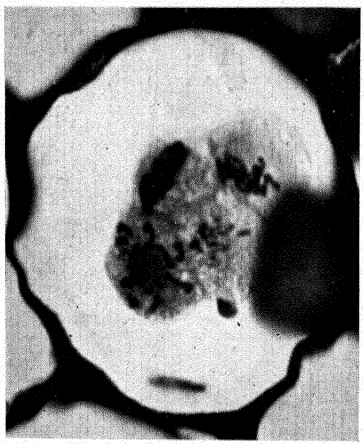
the fertilized egg. The cells of the embryo which give rise to both somatic cells and to germ-cells are known as *stem-cells*, and the cells derived from the stem-cells and from which the oogonia, or spermatogonia, originate are called the *primordial*, or *primitive*, *germ-cells*. The primitive germ-cells, after a period of multiplication, cease to divide, move to the position of the future gonads and give rise to the reproductive cells and to certain accessory cells of the ovary or the testis. In vertebrates the primitive germ-cells arise at an early stage of the embryo and migrate to the site of the gonads. There has been considerable controversy, however, as to whether the definitive ova and spermatozoa of vertebrates originate from the primitive germ-cells or from other sources.

In *Ascaris equorum* a stem-cell can be distinguished at the two-cell stage. During the prophase of the second-cleavage division the ends of the chromosomes of one of the blastomeres are broken off as large pieces while the central region breaks up into a number of small portions. The ends of the chromosomes move out into the cytoplasm and finally degenerate. The small pieces, derived from the central region of the chromosomes, divide longitudinally and, during the anaphase and telophase, the products of division are distributed to the daughter cells. The two new cells, therefore, contain a large number of small chromosomes which are visible at all the subsequent divisions of the cells which originate from these two blastomeres. The elimination of parts of the chromosomes is called *chromatin diminution*. The two cells which result from the division of the other blastomere of the two-cell stage contain the diploid number of large chromosomes (fig. 42, a and b), but one undergoes chromatin diminution and gives rise to somatic cells only. The process is repeated at the subsequent cleavages, and at the thirty-two-cell stage the cell which does not undergo diminution divides to form two cells like itself; these are the primordial germ-cells, and after a quiescent period they multiply to form the cells of the gonads (fig. 42, c). In *Ascaris equorum*, therefore, only the germ-cells contain the total amount of chromatin present in the fertilized ovum. The somatic cells are derived from the cells of the early cleavage stages which undergo diminution and contain a large number of small chromosomes.

Boveri produced experimental evidence to show that the factors which determine whether a cell gives rise to germ-cells or to somatic tissue only is located in the cytoplasm of the egg of *Ascaris*. Additional evidence in favour of this view has been obtained by the examination of the eggs of certain Diptera and of other invertebrates. In *Drosophila* and *Chironomus* (fig. 43), for example, granules are present at the posterior pole of the egg. One of the nuclei formed at the second mitosis migrates to the vicinity of the granules. It divides and the granules are included in the resulting two cells; the latter are extruded from the larger part of the embryo which forms the somatic tissues, and are the primitive germ-cells.



a



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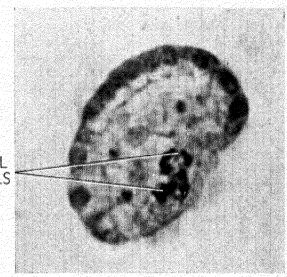


c

FIG. 42.—*Ascaris equorum*. Photomicrographs. a, two cells from an early cleavage stage showing the large chromosomes. One of these cells will undergo chromatin diminution. b, four cells from an early cleavage stage. In one cell the ends of the chromosomes are moving out into the cytoplasm ; the middle region of the chromosomes have broken up to form a number of small chromosomes. In another cell the chromosomes have not broken up. In the other two cells shown chromatin diminution has taken place. c, section of a gastrula showing the two primordial germ-cells. × 640.

In *Miastor* one of the first four nuclei moves to the posterior pole of the egg and divides to give one somatic cell and one *pole cell*, the latter receives a mass of deeply stained "*pole plasm*" which is previously present in the egg. At the third and fourth divisions chromatin diminution occurs, so that only the pole cell and the germ-cells, to which it gives rise, contain

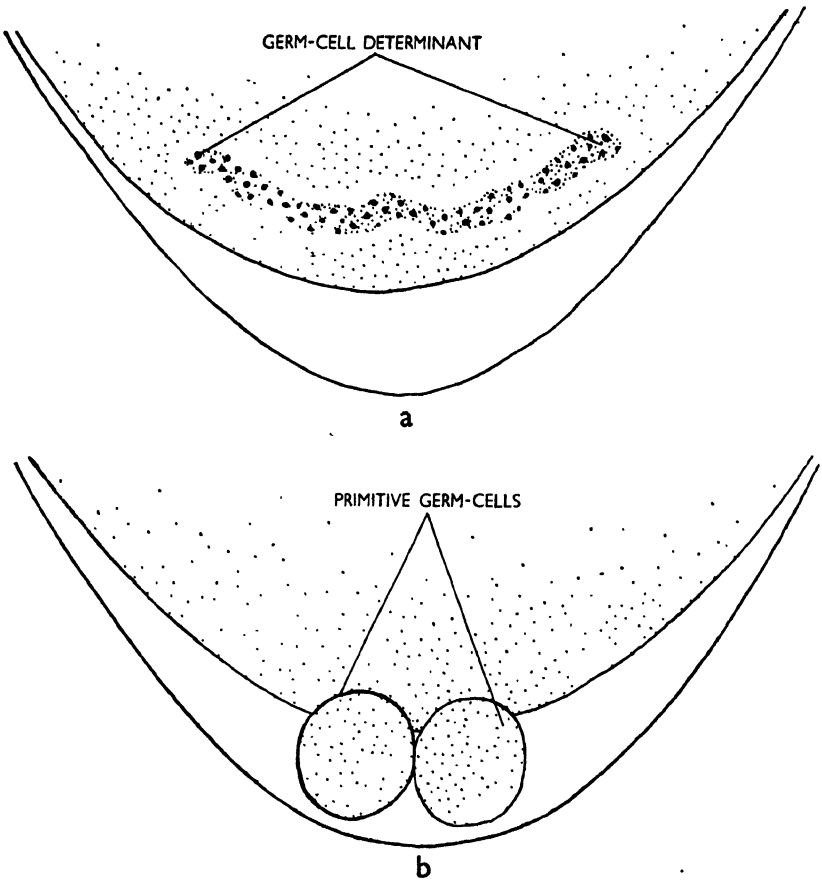


FIG. 43.—Egg of *Chironomus*. a, posterior part of egg showing germ-cell determinant. b, posterior part of egg showing the two primordial germ-cells. After Hasper, redrawn and modified.

the total amount of chromatin present in the nucleus of the fertilized ovum. Substances, in the form of deeply stained granules or cytoplasm, which are included in the primitive germ-cells, have received the name of *germ-cell determinant*. It has been shown that if the germ-cell determinant of certain beetles is destroyed by passing a hot needle into the egg, then development is normal up to a certain stage, but germ-cells cannot be identified. It is probable that the visible "germ-cell determinants" are not the true determining substances, but are the accompaniments of factors present in the cytoplasm

CHAPTER IX

REPRODUCTION IN PLANTS: I. THALLOPHYTA

WITH the exception of certain Thallophyta, the reproductive processes of plants are characterized by a well-marked alternation of a sporophytic generation producing spores and a gametophytic generation bearing gametes. Reduction of the chromosome number normally takes place during sporogenesis, so that the gametophyte arising from the germination of the spores is haploid. Gametes are formed without further alteration of the chromosome number, and their union results in the restoration of the diploid condition in the zygote. From this, the new sporophytic generation develops.

In spite of a general adherence to the above plan, there exists, however, among plants, a great diversity of reproductive phenomena, and in this and the succeeding chapters some of these will be discussed. Examples will be taken from the major divisions of the plant kingdom, *e.g.* the Thallophyta (Algae and Fungi), the Bryophyta (Hepaticae and Musci), the Pteridophyta (Filicales, Equisetales and Lycopodiales) and finally the seed-bearing plants, the Spermaphyta (Gymnosperms and Angiosperms).

THALLOPHYTA

Apart from the unicellular species, the plants of this group possess a plant body which is typically a *thallus*, a relatively undifferentiated plate or filament of cells, with chromatophores in the case of the Algae, and without chromatophores in the case of the Fungi. Reproduction is either *asexual* by means of spores, or *sexual* by the union of gametes. The latter often results in the formation of a resistant resting spore.

ALGAE

VEGETATIVE REPRODUCTION.—Vegetative reproduction may take place by means of cell division in the unicellular forms (Protococcales, Desmidiaceae, Bacilliariophyceae), multiplication of cells of the filament and subsequent fragmentation in filamentous forms (Zygnemaceae) or by detachment of portions of the thallus in the thalloid forms (*Dictyota*,

Fucus). In addition, certain filamentous Algae (*Cladophora*, *Pithophora*, etc.) produce specialized reproductive units, the *akinetes*, formed by the modification of complete cells, including the cell walls. These store food reserves of oil and starch, and help the plant to survive periods unfavourable for normal vegetative growth.

ASEXUAL REPRODUCTION.—Asexual reproduction is typically by means of motile *zoospores* (fig. 44, a and b), naked flagellated protoplasts, which arise within a *zoosporangium*. This structure may be simply an unmodified vegetative cell (*e.g.* Cladophoraceae) or a specialized reproductive organ (*e.g.* Phaeophyceae). One zoospore only may be liberated from the zoosporangium (*e.g. Oedogonium*) or, more frequently, by division of the contents, two, four—or even several hundreds may be set free. The whole of the protoplast is not always used in the formation of zoospores. Often the peripheral hyaline portion becomes mucilaginous, and by absorbing water and swelling, exerts pressure which breaks the wall of the zoosporangium at its weakest point, thereby releasing the reproductive cells. Each zoospore may possess two, four or many flagella attached to the narrow anterior end, one or more chloroplasts and frequently two contractile vacuoles and a red pigmented eye-spot. After a short period of activity, the zoospores withdraw their flagella, secrete a cellulose cell wall and eventually develop into a new plant.

Other asexual spores have been recognized in the Algae, *e.g.* non-motile *aplanospores*, which are regarded as zoospores which have lost the motile phase, and thick-walled, non-motile resting spores, the *hypnospores*.

SEXUAL REPRODUCTION.—Sexual reproduction may be either *isogamous*, by the fusion of morphologically identical gametes, or *heterogamous* (*oogamous*) by the fusion of unlike gametes. Heterogamous sexual reproduction seems to be associated with greater vegetative differentiation.

ISOGAMY.—The gametes arise by the division of the protoplasmic contents of a cell and are typically flagellate, motile, smaller than zoospores, invariably uninucleate and, except in certain species of *Chlamydomonas*, without a cell wall (fig. 44, c). Many of the isogamous Algae are dioecious and produce gametes of different sex types. Only gametes of different sex types conjugate, giving rise to diploid zygotes. In *Chlamydomonas* the diploid zygote forms a thick wall and becomes a zygote cyst. The nucleus undergoes two meiotic divisions and four flagellated haploid daughter cells are produced—in dioecious strains, two of one sex and two of the other. In certain *Chlamydomonas* species and varieties, Moewus has demonstrated the presence of a series of types possessing different sexual potencies. He assigns valencies from 1 to 5 to lines which occur among both males and females, and shows that conjugation may occur between members of the same sex showing sufficient difference in valency, the less extreme showing the reaction of the other sex. The work of Moewus, Kuhn and others on sexuality in *Chlamydomonas* is reviewed by Beadle (1945).

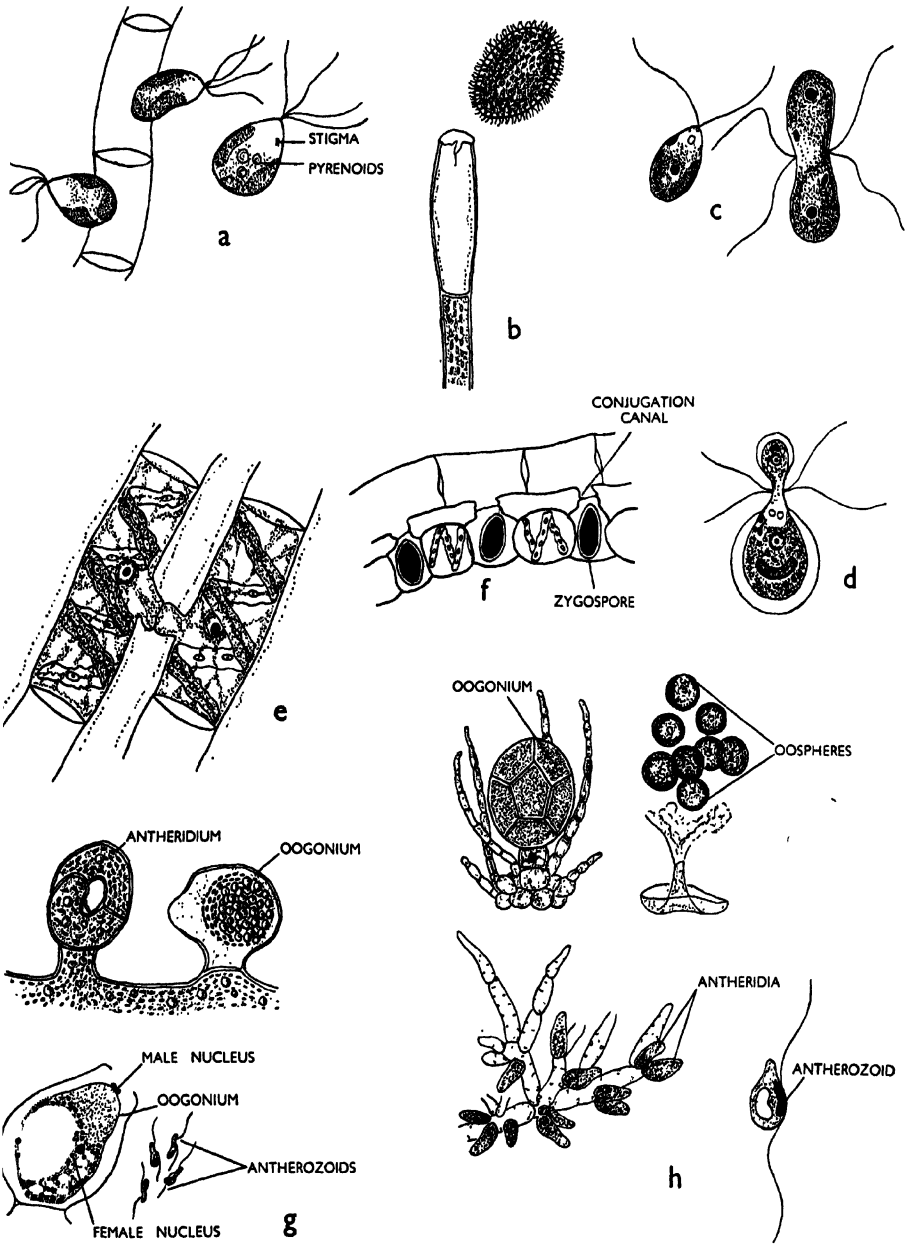


FIG. 44.—Reproduction in the Algae. a, quadriflagellate zoospores in *Ulothrix* showing eyespots (stigmata) and pyrenoids. After West, redrawn. b, multiflagellate zoospores of *Vaucheria*. After Goetz, redrawn. c, gamete and gametic fusion in *Chlamydomonas Reinhardi* Dang. After Goroschankin, redrawn. d, gametic fusion in the anisogamous *Chlamydomonas Braunii* Gorosch. After Goroschankin, redrawn. e, conjugation in Spirogyra showing association of adjacent filaments. After Coulter, redrawn. f, completed conjugation in *Spirogyra varians* Hass. showing conjugation canal and zygospore. After Czurda, redrawn. g, sexual reproduction in *Vaucheria kamata* Lyngb. showing antheridium and oogonium prior to fertilization, and oogonium and antherozoids at fertilization with female and male nuclei. After Oltmanns, redrawn. h, sexual reproduction in *Fucus vesiculosus* L. with oogonium, liberated oospheres, antheridia, and biflagellate antherozoid. After Thuret, redrawn.

Cases of *anisogamy* in Chlamydomonas, where slight differences in size and behaviour can be observed between the gametes (fig. 44, d), and where one, usually the larger and more passive, receives the entire cell contents of the other, clearly represent a step towards true heterogamy in which a large non-motile oosphere is fertilized by a small active antherozoid.

Physiological anisogamy is also encountered in *Spirogyra* and certain species of *Zygnema*. In *Spirogyra* union takes place between cells of adjacent filaments (fig. 44, e and f). The entire protoplast of one conjugating cell contracts away from the wall and becomes the amoeboid male gamete. This passes through a conjugation tube to the female gamete, but nuclear fusion seems to be delayed until some time after the union of the gametes. The movement of the male gamete is thought to be brought about by the action of contractile vacuoles which withdraw water from a central vacuole and discharge it between protoplast and cell wall. Thick-walled *zygospores* are formed in which only the female chloroplast can be recognized. The male chloroplast disintegrates at an early stage in the process of conjugation.

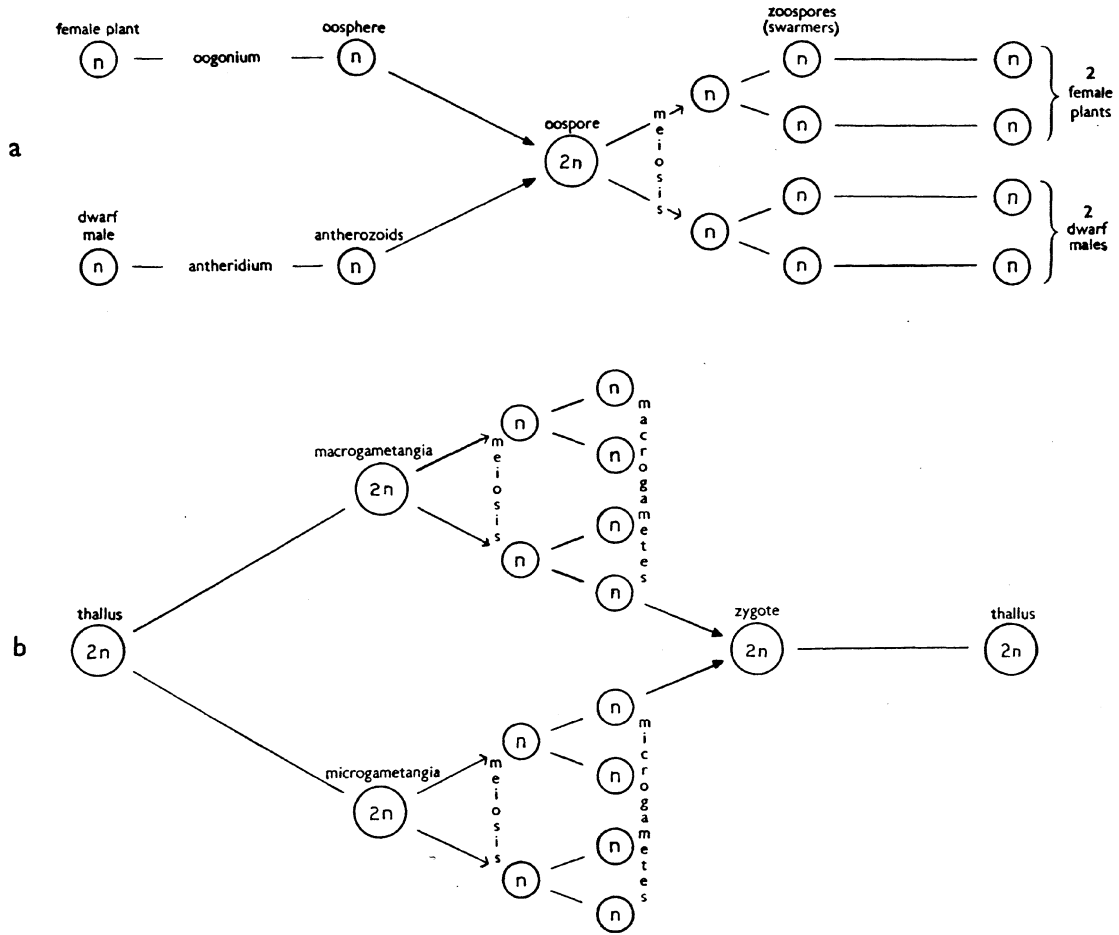
Some species of *Zygnema* and all the *Debarya* are truly isogamous. The gametes are neither morphologically nor physiologically differentiated and zygospores are formed within the conjugation canal.

HETEROGAMY.—In the heterogamous Algae specialized reproductive organs are formed, clearly distinguishable from the ordinary vegetative cells. The female organ is the *oogonium* which, with the exception of *Fucus* and certain other Brown Algae, develops only one *oosphere* with one or more chromatophores and abundant food reserves. The male organ, the *antheridium*, gives rise to many *antherozoids* which, except in the Rhodophyceae, are active and flagellate. The male gametes are without food reserves and never contain more than one small and inconspicuous chromatophore. A resting spore, the *oospore*, results from the union of male and female gametes (fig. 44, g and h).

Usually the oosphere is retained within the oogonium but in the Phaeophyceae it is released prior to fertilization. The antherozoid may penetrate the oogonium through an aperture in the wall from which protoplasmic slime exudes (*Oedogonium*, *Vaucheria*), and a clear area in the cytoplasm, often observed near this aperture, is known as the *receptive spot*. Both monoecious and dioecious species occur.

MEIOSIS IN THE ALGAE.—The stages in the life-histories of the Algae at which reduction of the chromosomes occurs, vary considerably, but, broadly speaking, four different groups can be recognized (fig. 45, a, b, c and d).

(a) *The Haplonts*.—In this group the organism is haploid and only the zygote, as a result of sexual fusion between the gametes, has the diploid chromosome number. This state of affairs is found in the majority of Chlorophyceae and, according to Fritsch (1935), probably also in all the Xanthophyceae and Chrysophyceae. Meiosis takes place during the


a
female plant
n
oogonium
oosphere
n
dwarf male
n
antheridium
antherozoids
n
oospore
2n
meiosis
zoospores (swarmers)
n
n
n
n
2 female plants
n
n
n
n
2 dwarf males
b
thallus
2n
macrogametangia
2n
meiosis
n
n
n
n
macrogametes
microgametangia
2n
meiosis
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n
n
n
microgametes
zygote
2n
thallus
2n

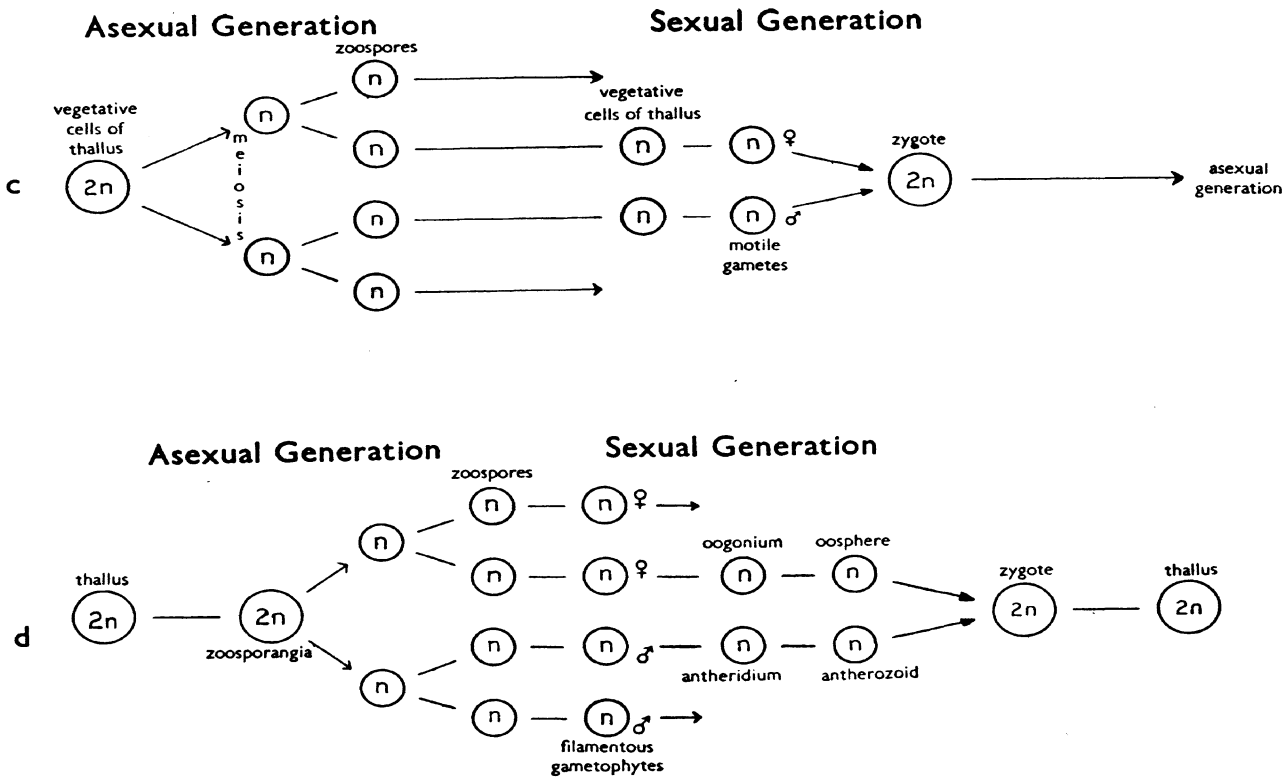

FIG. 45.—a, life cycle of a typical *Haplont, e.g.* the dioecious *Oedogonium plagiostomum var. gracilis.* b, life cycle of a typica *Diplont, e.g. Codium tomentosum* Huds. c, life cycle of a typical *Diplohaplont, e.g.* the dioecious *Enteromorpha intestinalis* L d, life cycle of *Chorda filium* L. showing well-marked antithetic alternation between a diploid sporophytic and a haploi gametophytic generation.

first two divisions of the zygote, and these divisions may be followed or accompanied by division of the protoplast to form four asexual cells which can be liberated either as zoospores (*e.g. Oedogonium*) or as aplanospores.

(b) *The Diplonts.*—Here the normal vegetative phase is diploid, and meiosis occurs during gametogenesis. Only the gametes, therefore, are haploid. Diplonts are comparatively rare, but examples are found in the Siphonales (Chlorophyceae), the Diatoms (Bacilliariophyceae) and the Fucales (Phaeophyceae).

(c) *The Diplohaplonts.*—In this case there are two well-marked but morphologically identical generations, in which plants with the haploid chromosome number bear the gametes and those with the diploid, the asexual spores. Meiosis occurs during zoosporogenesis. Organisms showing this *isomorphic* or *homologous* alternation are found in the Cladophorales and Ulvaceae (Chlorophyceae), in the Dictyotales and Cutleriales (Phaeophyceae) and in the majority of the Rhodophyceae. In this last group, however, two diploid phases alternate with one haploid. One of the asexual diploid stages bears *tetraspores*, during the formation of which reduction occurs. These germinate to produce a morphologically identical haploid organism, bearing the sexual reproductive organs. The zygote (the fertilized *carpogonium*) gives rise to one or more threads, the *gonimoblasts*, which ultimately produce asexual *carpospores*. These, in turn, germinate to produce the diploid tetrasporic plant.

(d) In many of the Brown Algae (Phaeophyceae) a well-marked heteromorphic or *antithetic* alternation occurs between a comparatively large diploid sporophytic generation and a smaller haploid gametophytic generation. This is very pronounced in the Laminariales where the large many-celled sporophyte is in great contrast to the much smaller gametophyte. Meiosis occurs during the first two divisions in the sporangium, and the motile zoospores of any one sporangium give rise to male and female gametophytes in equal numbers. Sex determination must therefore take place at reduction. Such forms are exceedingly interesting, for they demonstrate that the basis for the evolution of true antithetic alternation, such as is found in the higher plants, exists in the Algae.

FUNGI

VEGETATIVE REPRODUCTION.—Vegetative reproduction may be by means of the budding of a single fungal cell, as in *Saccharomyces* and the torula stages of *Mucor*, *Penicillium* and *Eurotium*, or by the compacting of the fungal hyphae into *sclerotia* as in *Sclerotium rolfsii* or into the *rhizomorphs* of *Armillaria mellea.* In each of the last two structures the hyphal core is preserved from desiccation by the modification of the outer layers into a hard, resistant covering.

ASEXUAL REPRODUCTION.—Asexual reproduction takes place by means of spores produced either *endogenously* (*i.e.* within a sporangium) or *exogenously* (by abstriction from a hypha). In the first case the spores can be either motile and flagellate, as in *Pythium*, *Saprolegnia*, *Leptolegnia* and *Synchytrium* (Oomycetes), or non-motile as in *Mucor* and *Rhizopus* (Zygomycetes). Both *Saprolegnia* and *Leptolegnia* illustrate very clearly the phenomenon of *diplanetism*, met with in certain aquatic Phycomycetes, in which the zoospores undergo two periods of active movement separated by an encysted stage.

Asexual reproduction by means of *conidia* is widespread. Conidia arise exogenously, usually by successive abstrictions from the ends of branched or unbranched *conidiophores*, which may be scattered and exposed or enclosed in flask-shaped receptacles, the *pycnidia*. Under certain conditions hyphae may break into segments which, rounding off and separating from one another, function as asexual spores in the manner of conidia. Spores formed in this manner are frequently termed *oidia*.

Also formed by modification of part of the vegetative hyphae in many of the mycelium-producing fungi are the thick-walled *chlamydospores*, which serve, by virtue of a relatively copious food store, to carry the species through periods unfavourable to the growth of the mycelium or to the survival of the normal reproductive bodies.

It must be emphasized at this point that zoospores, conidia, oidia and chlamydospores are not homologous with the spores of the Bryophyta and Pteridophyta and have no significance in the alternation of generations. They may be borne either on the sporophyte as in the Rust Fungi and Autobasidiomycetes, or on the gametophyte as in most Phycomycetes and Ascomycetes. They serve merely as a rapid means of multiplication and dissemination, and for this reason have been termed *accessory spores* (Gwynne-Vaughan). The characteristic spores of the sporophyte, with the development of which the reduction division is associated, are the ascospores and basidiospores. The significance of these bodies in the life-histories of the higher Fungi will be discussed subsequently.

SEXUAL REPRODUCTION.—As a consequence of the fact that, in the Fungi, there is a strong evolutionary tendency towards the disappearance of the sexual process, resulting in great reproductive diversity, it is not easy to treat as a whole the sexual reproductive processes in this group. Each of the main sections will therefore be considered separately.

PHYCOMYCETES.—Within the Phycomycetes considerable variation in sexual behaviour occurs.

In *Synchytrium endobioticum* (Archimycetes) isogamous union between pairs of similar motile, uniflagellate gametes results in the formation of naked biflagellate zygotes. These gametes seem to be zoospores which have been retarded in their development by adverse conditions,

and which in more favourable circumstances would have behaved as asexual spores.

A peculiar state of affairs exists in *Allomyces javanicus* (Kniep, 1929–1930, 1930) where resting spores, particularly after a period of desiccation, are formed directly into resting sporangia. These sporangia eventually produce uniflagellate zoospores, which in turn germinate to give plants bearing chains of *gametangia*. The gametangia are of two sizes; the larger release relatively large, colourless, uniflagellate gametes, while the lesser set free smaller gametes, each characterized by a conspicuous orange globule. Dissimilar gametes fuse in pairs, and the resultant biflagellate zygote germinates almost immediately, to give a thallus identical with that producing gametangia, but bearing zoosporangia and resting spores. It is believed that this thallus is diploid and that meiosis occurs prior to the

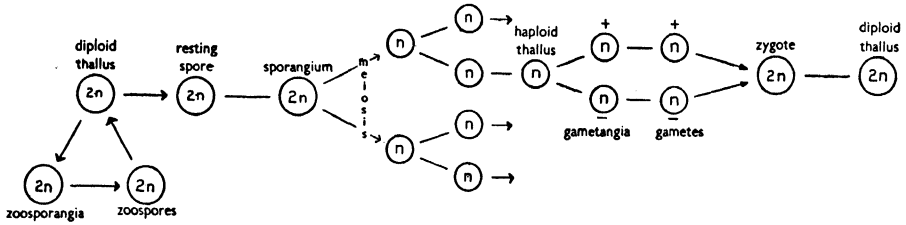

FIG. 46.—Life cycle of *Allomyces javanicus* Kniep.

formation of the motile spores in the resting sporangia. The plants bearing gametangia which arise from these spores will therefore be haploid, and there is thus an alternation between diploid and haploid generations (fig. 46).

Union between a non-motile uninucleate *oosphere*, developed within an oogonium, and a small motile male gamete takes place in *Monoblepharis sphaerica* (Oomycetes) (fig. 47, a). The male organs, or antheridia, originally contain five or six nuclei, around each of which a male gamete is organized. These are released as uniflagellate antherozoids which swim towards the oogonium, where they move for some time on its surface in an amoeboid manner Only one takes part in the fertilization of the oosphere. The oosphere, after penetration by the male gamete, frequently leaves the oogonium and matures outside. The resultant oospore develops a thick coat and germinates after a period of rest. Meiosis is believed to occur during the early stages of the germination of the oospore.

In other genera of the Oomycetes (*e.g. Pythium*, *Albugo*, *Peronospora*) the antheridium gives rise to a single amoeboid male gamete which is transferred to the oogonium by means of a conjugation tube. Typically, both oogonium and antheridium are originally multinucleate. As the organs ripen, the contents become differentiated into single gametes and surrounding periplasm (fig. 47, b). During this process, the nuclei pass to the periphery and there divide once, after which, in both antheridium

and oogonium, all but one degenerate. The surviving nuclei become, respectively, the functional male and female nuclei. Fusion of the gametes results in the formation of a thick-walled resting spore, the oospore.

In the Zygomycetes, of which *Mucor*, *Rhizopus* and *Sporodinia* are typical examples, sexual fusion occurs between similar multinucleate gametangia, producing a thick-walled zygospore. Gametangia may develop without fertilization into *azygospores*. It was early observed that whereas in some species, *e.g. Sporodinia grandis*, zygospores were produced in experimental cultures without difficulty, in other cases, *e.g.*

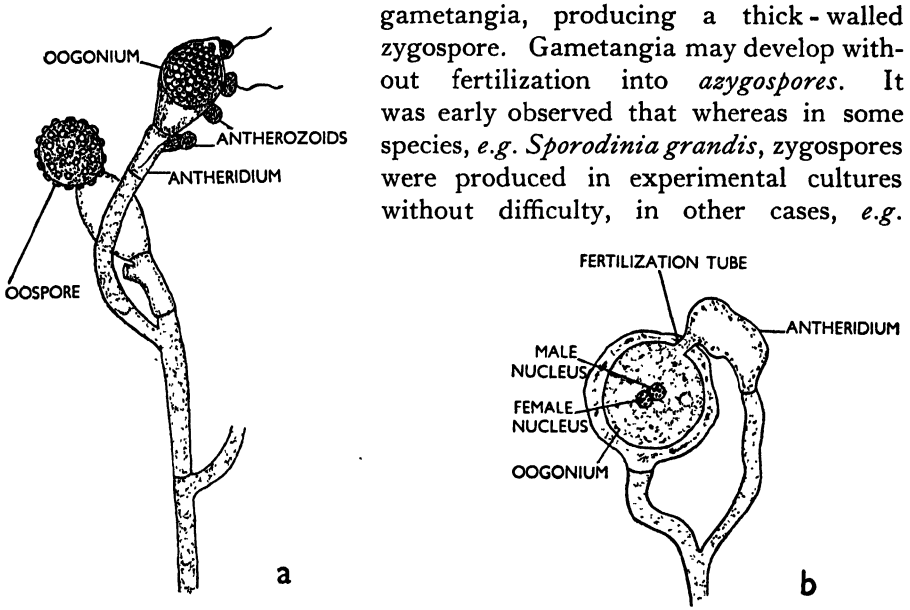

FIG. 47.—a, *Monoblepharis sphaerica*, showing fertile branch bearing oogonium, antheridium with emerging antherozoids, and oospore. After Woronin, redrawn. b, *Pythium de Baryanum* Hesse. Oogonium and antheridium immediately after fertilization showing fertilization tube and male and female nuclei. After Miyake, redrawn.

Rhizopus nigricans, zygospore production occurred only sporadically. Species such as *Rhizopus nigricans* are *heterothallic*, *i.e.* they consist of two strains, each of which when grown apart produce only sporangia, but when grown together produce zygospores. To these two strains the terms (+) and (−), or (A) and (B), have been applied. Species such as *Sporodinia grandis*, in which zygospore formation occurs with ease upon the thallus developed from the culture of a single spore, are said to be *homothallic*.

In some of the heterothallic Mucorales no visible difference between the strains can be detected, though a physiological one obviously exists. In others, one strain, designated the (+), is the more luxuriant.

Union of the gametangia of the heterothallic *Mucor mucedo* is followed by the fusion of the nuclei in pairs and the development of a thick-walled zygospore. This eventually germinates to produce a *promycelium* bearing

an apical sporangium, the spores from which produce mycelia which are either all (+) or all (−). In *Phycomyces Blakesleeanus*, on the other hand, the germinating zygospore gives rise to a sporangium containing both (+) and (−) spores as well as spores which produce weak-growing homothallic mycelia. The nuclear phenomena which lead up to strain differentiation in these two species are exceedingly difficult to follow, but it seems likely that in *Mucor* differentiation occurs during meiosis, which is believed to take place in the zygospore prior to its germination, while in *Phycomyces* the process must be delayed until a later stage.

ASCOMYCETES.—The typical spore of the Ascomycetes is the *ascospore* produced in limited numbers within a specialized mother cell, the *ascus*. The ascospore, on germination, gives rise to a mycelium which, in those forms with normal sexual reproduction, produces the sexual organs. These consist of an antheridium, borne as an antheridial branch, and an *archicarp* consisting of a stalk, oogonium and an apical fertilization tube, the *trichogyne*. The oogonium may be simply a single uninucleate cell as in *Erysiphe*, a multinucleate hyphal segment as in *Eurotium* or a multinucleate pear-shaped structure such as the oogonium of *Humaria granulata* which may contain as many as 1400 nuclei. The antheridium, too, may possess one or many nuclei. After the entry of the antheridial nuclei, the oogonia often become septate and filaments known as ascogenous hyphae are produced. These eventually give rise to the asci, in the first two divisions of which reduction takes place. The ascogenous hyphae, therefore, represent the diplophase or sporophyte, whereas the vegetative hyphae bearing the sexual organs represent the haplophase or gametophyte (fig. 48). The gametophyte may also bear accessory spores, such as conidia or chlamydospores, which serve rapidly to reproduce the haploid phase.

Although the occurrence of sexual organs in the Ascomycetes was first reported by de Bary and others during the latter half of the nineteenth century, and nuclear fusion in the ascus of *Peziza vesiculosa* was observed by Dangeard in 1894, the details of the sexual processes are still not entirely clear.

In 1895 the life cycle of the Hop Mildew, *Sphaerotheca Humuli*, was studied by Harper. Here, as in other species of Ascomycetes which have been investigated more recently, both antheridium and oogonium are uninucleate, and nuclear fusion takes place within the oogonium. The fertilized oogonium becomes septate, giving rise to a row of cells, of which the subterminal cell is binucleate and becomes the ascus (fig. 48). As the ascus develops, the two nuclei fuse to give the *primary ascus nucleus*, which is therefore tetraploid. It has furthermore been shown that the primary ascus nucleus subsequently undergoes three successive divisions, during the first two of which reduction takes place. The third division is also believed to involve a reduction in the chromosome number, which is

known as *brachymeiosis* (Tandy, 1927; Gwynne-Vaughan and Williamson, 1930, 1931, 1932, 1933, 1934). In those species in which antheridia and oogonia are not normally produced, the ascogenous hyphae may arise directly from vegetative cells with haploid nuclei. Here, again, the ascus is originally binucleate, and nuclear fusion is followed by three successive divisions of the fusion nucleus. In this case the third division appears to be purely mitotic and brachymeiosis does not occur.

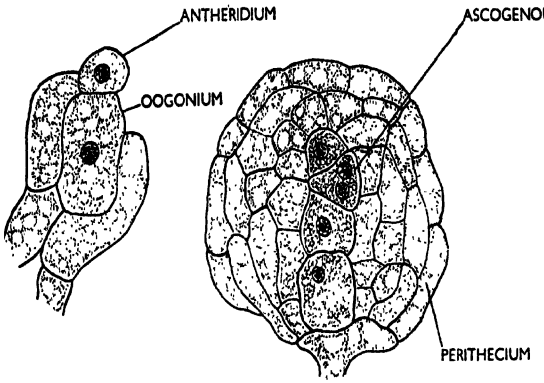

FIG. 48.—*Sphaerotheca Humuli* Burr. showing oogonium, antheridium, and young perithecium with binucleate ascogonous cell. After Blackman and Fraser, redrawn.

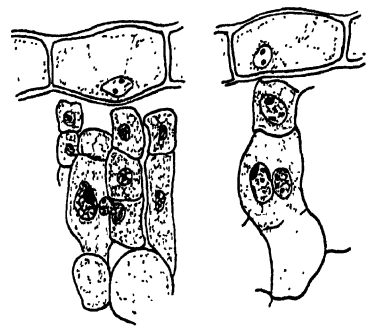

FIG. 49.—*Phragmidium violaceum* Wint. showing migration of nucleus from vegetative cell of hypha to neighbouring fertile cell. After Welsford, redrawn.

Although the above course of events is undoubtedly followed in certain species of Ascomycetes, cases (*e.g. Peziza, Pyronema*) have been investigated where fusion between male and female nuclei within the oogonium does not occur. The two nuclei remain associated and divide repeatedly in pairs. Fusion finally takes place within the developing ascus, the primary nucleus of which is therefore diploid and not tetraploid. Reduction in chromosome number takes place during the first of the three divisions which follow.

It is interesting to note in this connection that in the fungus *Neurospora sitophila* segregation of Mendelian factors, and therefore, one deduces, of homologous chromosomes, occurs during the first or second division of the primary ascus nucleus, but not in the third.

BASIDIOMYCETES.—Sexual reproductive organs are only found in one group of the Basidiomycetes, the Uredinales of the Protobasidiomycetes, to which the Rust Fungi belong. Although possessing such organs, the normal union of male and female nuclei does not occur, but is replaced by a nuclear association giving rise to a mycelium of binucleate cells, the paired nuclei of which are known as a *dikaryon*. This association ends in the basidium where fusion between the paired nuclei is immediately followed by meiosis.

In the non-sexual Basidiomycetes the young basidium and some part of the mycelium are always binucleate, and here, again, nuclear fusion occurs in the basidium. The binucleate condition in the Hemibasidiomycetes may arise from the fusion of basidiospores, while in the Autobasidiomycetes the germination of the basidiospore may give rise to a mycelium of uni- or multinucleate cells, between which anastomoses are common and result in the development of the dikaryon.

A complete account of the variation of behaviour which is found in the Uredinales alone would be impossible here and the student is referred to the relevant literature. For the following summary I am greatly indebted to *The Structure and Development of the Fungi* by Gwynne-Vaughan and Barnes (1937).

The basidium or *promycelium* arises as the result of the germination of a *teleutospore* which may consist of one, two or more cells, originally binucleate. Following the fusion of the two nuclei, the cells pass into the resting state, but on the renewal of activity the fusion nucleus of each divides twice (meiosis), the daughter nuclei become separated by cross walls, and a separate basidium is formed. From each basidial cell a pointed sterigma is protruded, forming at its apex a single basidiospore or *sporidium*. Into this the nucleus and cytoplasm of the parent cell pass and the basidiospore thus becomes the first stage in the haploid phase.

The germination of the basidiospore on a suitable host produces a uninucleate or occasionally multinucleate mycelium, bearing *spermagonia* and *aecidia*. The flask-shaped spermagonia are usually found on the upper surface of the leaf of the host plant and consist of groups of *spermatial hyphae* surrounded by sterile paraphyses. From the spermatial hyphae a succession of thin-walled, ovoid, uninucleate *spermatia* are abstricted.

The aecidia are cup-shaped structures occurring in clusters on the under surface of the leaf. The individual aecidium develops from a group of hyphae, each of which cuts off a terminal sterile cell and a larger *oogonium*, or *fertile* cell. These fertile cells may unite laterally in pairs, fuse with the cells directly below them, or receive a second nucleus by migration from a neighbouring vegetative cell. In each case, however, they proceed immediately to cut off binucleate *aecidiospore mother cells*, which in turn divide to form an upper *aecidiospore* and a lower intercalary cell. The fertile cells are thus the first stage in the diploid phase (fig. 49).

Contrary to former belief, it now seems highly probable that in the Rust Fungi the spermatia are functional and may induce the formation of the binucleate aecidiospore chains. Thus, in *Uromyces appendiculatus*, Andrus (1931) has observed the passage of spermatia through the tips of receptive hyphae (trichogynes) which emerge through stomata or between epidermal cells of infected plants. This passage is followed by the appearance of binucleate cells in the aecidial primordium. Similarly in *Puccinia*

triticina, Allen (1932) reports that from a single basidiospore, spermatia, receptive hyphae, and aecidial primordia arise, but only after the contents of spermatia of the opposite strain enter the receptive hyphae do binucleate aecidiospores result.

When able to germinate on the appropriate second host, the aecidiospores produce a mycelium of binucleate cells from which the binucleate *uredospores* arise. These accessory spores serve to propagate this diploid stage in the life-history of the fungus, but eventually teleutospores replace the uredospores and the life cycle is completed.

HETEROTHALLISM IN THE ASCOMYCETES AND BASIDIOMYCETES.—The phenomenon of heterothallism, which has already been mentioned in connection with certain of the Phycomycetes, occurs commonly in the Ascomycetes and Basidiomycetes. It has been defined by Gwynne-Vaughan as " the existence in a given species of more than one gametophytic strain or thallus, two of which must come together to bring about or expedite the formation of the diplophase ".

In the Ascomycetes the true dioecious condition is rarely encountered, but in the monoecious species heterothallism may take one or two forms. Thus, in the self-sterile *Ascobolus magnificus*, A and B strains, morphologically indistinguishable, can form both antheridia and oogonia, but the sexual organs are only differentiated when the strains are in contact. Reciprocal fertilization takes place between antheridia of A and oogonia of B and between antheridia of B and oogonia of A. The sporophyte therefore carries both A and B nuclei and produced both A and B spores. The release of these together ensures that the two strains will grow closely intermingled. In the self-incompatible *Humaria granulata*, on the other hand, both A and B strains produce only the female organs, and in the absence of antheridia normal fertilization is impossible. Mycelial fusion between complementary thalli, however, stimulates the female organ to develop further, and presence of both A and B producing spores in the resulting asci shows that nuclei from both strains have taken part in their formation. A variation of this procedure is found in *Pleurage anserina*. Here only four ascospores are produced, each of which is binucleate. These germinate to produce homothallic or self-compatible mycelia bearing sexual organs of both types. Occasionally, however, small uninucleate spores occur, which on germination produce two types of mycelia, self-sterile but mutually compatible. Ames has shown that incompatibility is inherited in a Mendelian fashion, but there is no segregation of sex-factors as such, as each strain is capable of producing both male and female organs.

Similar phenomena are met with in the Basidiomycetes but the position is complicated by the existence of *multipolar* species. Thus, in one of the Smut Fungi, *Ustilago longissima*, there are three strains, each self-sterile but fertile with either of the other two. *Coprinus lagopus*, amongst other

species, is tetrapolar, having four strains, AB, Ab, aB and ab, the diplophase or *secondary mycelium* only arising as a result of a combination giving AaBb.

In such cases it has been suggested that the sporophyte is heterozygous for two pairs of factors controlling incompatibility. The position is further complicated by the occurrence of local races carrying different allelomorphs of A and B so that additional fertile combinations are possible.

Some information concerning the nature of these factors is available. In certain species of *Ustilago* the A factor is a sterility or lethal factor, the homozygote being unable to survive. The B factor, on the other hand, controls conjugation, which only takes place when the B factors of the two mycelia are different

In many species of the Uredinales, aecidia are formed in infections arising from a single basidiospore, but in others monosporidial infections produce only spermagonia. In these latter cases the production of aecidia seems to be dependent on contact with a complementary strain, aecidial formation being induced either by the passage into the fertile cell of a nucleus from a vegetative cell of the opposite strain or by the presence of complementary spermatia.

The effectiveness of heterothally as an outbreeding mechanism in the Fungi has been discussed by Mather (1942), and the student is referred to this review for further information on the subject

CHAPTER X

REPRODUCTION IN PLANTS: II. BRYOPHYTA AND PTERIDOPHYTA

BRYOPHYTA

THE Liverworts (Hepaticae) and Mosses (Musci) together form a distinct group of plants with a sharply defined alternation between gametophytic and sporophytic generations. In both groups the sexual generation is the more highly developed vegetatively, while the asexual, though usually possessing chlorophyll and therefore able to carry out carbon assimilation, has no separate existence and is dependent on the gametophyte for a large part of its nutrition. In both generations the mosses show greater differentiation than the Liverworts, but otherwise the life cycles of the two groups are identical and can be considered together.

VEGETATIVE MULTIPLICATION.—Both Liverworts and Mosses reproduce freely by vegetative means. Not only does a *protonema* develop readily from almost any part of the adult Moss plant, but most foliose Liverworts, some thalloid Liverworts and many Mosses produce specialized reproductive units, the *gemmae*. These consist of characteristically shaped groups of cells either borne in cup-like outgrowths on the upper surface of the thallus as in the Liverwort, *Marchantia polymorpha*, or budded off from various parts of the aerial shoots as in the leafy Liverworts and some Mosses.

SEXUAL REPRODUCTION.—The haploid gametophyte bears the sexual reproductive organs, antheridia and archegonia, the former producing motile antherozoids and the latter containing usually a single oosphere. Both monoecious species, *e.g. Funaria* spp. (Musci), *Pellia epiphylla* (Hepaticae) and dioecious species, *e.g. Polytrichum* spp. (Musci), *Pellia Neesiana* (Hepaticae), occur.

The archegonium (fig. 50, a) is a flask-shaped structure with an elongated neck, containing a row of *neck canal cells*, and a swollen basal portion, the *venter*, within which lies the oosphere. Above the oosphere is the smaller *ventral canal cell*, which in certain genera, *e.g. Sphagnum* (Musci) and *Anthoceros* (Hepaticae), is a functional egg-cell. There is some evidence that the neck canal cells, too, are degenerate female gametes. As the archegonium develops the neck canal cells disorganize and become mucilaginous. The mature oosphere is a large rounded cell, with a very

distinct nucleus, plastid primordia and mitochondria; in many cases a distinct transparent receptive spot can be seen on its upper surface.

The antheridium (fig. 50, b) arises from a single superficial cell, and when mature consists of a wall one cell thick surrounding a mass of spermatogenous tissue. During the divisions of the *androcyte mother cells* of the Liverwort, *Marchantia*, a small centrosome, apparently of nuclear origin, appears and divides into two. The two halves move apart and finally occupy positions at opposite poles of the mitotic spindle. Following the division, which differentiates the *androcyte* (spermatid), the centrosome passes to one corner of the cell and there forms an elongated

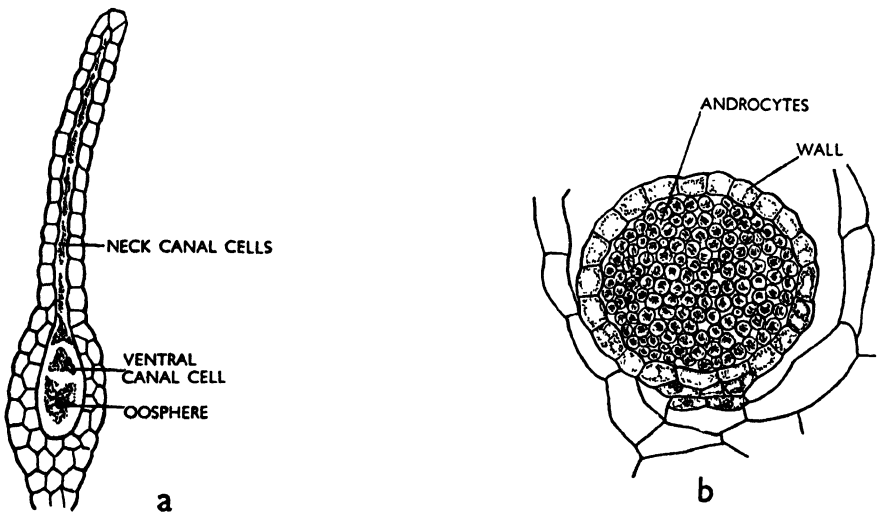

FIG. 50.—a, archegonium of the Moss, *Funaria*, showing degenerating neck canal cells, ventral canal cell and oosphere. b, antheridium of the Liverwort, *Pellia*, showing wall and androcytes. After Fritsch, redrawn.

blepharoplast from which two long slender flagella arise. The body of the mature antherozoid is almost entirely nuclear in origin. Variations of this process are seen in the Liverworts, *Blasia*, where the blepharoplast breaks up to form a flagella-bearing rod, and *Riccardia* and *Pellia*, in which the flagella arise from two distinct points, at each of which basal granules can be seen.

In the Moss, *Polytrichum juniperinum*, each androcyte contains a small spherical blepharoplast which gradually elongates to form a slender curved rod, from which, at a point a little distance from the anterior end, two long flagella emerge. At the same time the nucleus moves into contact with the blepharoplast and also elongates, becoming a long attenuated coiled body, hardly to be distinguished from the latter. The two organs together form the whole body of the mature antherozoid. In the meantime a large rounded body, the *limosphere*, appears in the cytoplasm and moves into

position close to the anterior end of the blepharoplast. Here it divides into two, the smaller portion becoming the *apical body*, which remains closely attached to the blepharoplast, although it has not been proved to take any part in the formation of the body of the antherozoid. The remaining part of the limosphere takes up a position near to the posterior end of the nucleus, but disappears before the antherozoid is mature.

The limosphere is derived from the plastids of earlier spermatogenous cells: this is of particular interest in view of the close relationship which seems to exist between the behaviour of the limosphere and apical body in *Polytrichum* and the Golgi material and acrosome in animal spermatogenesis (Chapter VII). The plastid origin of the limosphere is cited as evidence in favour of the view that the plastids of plants correspond to the Golgi material of animals.

When mature, the antherozoids swim towards the archegonium, are caught in the exuding mucilage, and gradually work their way down the neck. Fusion of male and female gametes initiates the sporophytic generation.

In *Riccardia pinguis* and *Pellia Fabbioniana* (Hepaticae) the spermatozoid attaches itself to the surface of the egg and undergoes an immediate reduction in thickness. Gradually the slender male nucleus passes through the egg membrane into the cytoplasm, and there remains unchanged for about ten hours. At the end of this period it approaches the female nucleus and abruptly alters its form, becoming shorter and thicker. As this change is taking place, vacuoles appear in the cytoplasm close to the male nucleus. These gradually enlarge and coalesce until one large vacuole completely surrounds the male nucleus, which at this stage is seen as a mass of chromatic material, the outer limit of which is not clearly defined. The granular cytoplasm between the two sexual nuclei recedes, leaving the male nucleus lying close to the membrane of the female nucleus. The membrane soon disappears at the point of contact and the two nuclei eventually become enclosed in a common membrane. It seems that, in some cases at least, a portion of the vacuole previously surrounding the male nucleus is left outside the fusion nucleus. The actual union of the two sexual nuclei occurs usually at from twenty-four to thirty hours after insemination of the female plants.

ASEXUAL REPRODUCTION.—From the fertilized ovum arises the sporophytic generation consisting of a conical *foot*, firmly embedded in, but not continuous with, the tissues of the gametophyte, the *seta* or stalk, and an apical *capsule*. Surrounding the young capsule is the *calyptra*, formed from the enlarged venter of the archegonium, although in some Liverworts part of the thallus may also be included in its structure. This organ is therefore gametophytic in origin. As the capsule matures, the single-layered archesporium divides repeatedly, giving rise to a mass of *spore mother cells* or *sporocytes*. By means of two successive divisions at which

reduction of the chromosome number occurs, a tetrad of spores is formed from each sporocyte. During these divisions the plastids of the sporocytes divide and are distributed amongst the young spores, in which they rapidly become typical chloroplasts.

The spores of most Bryophyta germinate to produce a branched filamentous *pro-embryo* or *protonema* on which the young gametophytic plants develop as lateral buds. In some thalloid Liverworts, however, the protonema takes the form of a discoid mass of cells, while the spores of *Pellia* are unique in that they become multicellular while still in the capsule.

INHERITANCE OF GAMETOPHYTIC CHARACTERS IN MOSSES.—Meiosis in the Bryophyta takes place in the capsule prior to the formation of the spore tetrad, and the spores give rise to haploid gametophytes. Homologous chromosomes separate from one another during meiosis and are distributed at random to the daughter cells, half of which will, therefore, carry one member of a pair and half the other (Chapter VI). As the chromosomes are the bearers of factors concerned with the transmission of hereditary characters, the study of haploid gametophytic plants gives a unique opportunity for the investigation of the mechanics of inheritance. To this end Wettstein crossed two races of the Moss *Funaria hygrometrica*, which differed in several respects, including the rate of growth of the protonema. If rate of protonema growth is controlled by a single pair of factors Gg, then the diploid sporophyte of the hybrid would be heterozygous and the spores of each tetrad would be of two kinds, two carrying the factor G and two the factor g. This was found to be the case, and the four spores of the tetrad, which remain associated, germinated to give two slow-growing and two quick-growing protonemata. Thus, segregation of the factors controlling the rate of protonema growth followed the segregation of chromosomes at meiosis. In addition, Wettstein was able to show, by studying the inheritance of more than one pair of gametophytic characters, that each tetrad always consists of spores of two types only, so demonstrating that segregation of the controlling factors takes place at the first of the two meiotic divisions. Had it taken place at the second, spores of four types would have been produced. Similar results have been obtained by Allen in his studies of the inheritance of gametophytic characters in the Liverwort, *Sphaerocarpos Donnellii*.

PTERIDOPHYTA

The Pteridophyta, of which there are three main groups, the Filicales (Ferns), Equisetales (Horsetails) and Lycopodiales (Club Mosses), differ from the Bryophyta in that, although there is an equally distinct alternation of generations, the emphasis is on the sporophyte. This, in all three sections, is a well-grown plant showing a high degree of tissue differentia-

tion. The gametophyte, in contrast, is small and inconspicuous, varying from the independent prothallus of the Ferns to the reduced condition encountered in *Selaginella*, where the male gametophyte consists of a single prothallial cell and a degenerate antheridium, and the female gametophyte, while slightly more complex, is never completely free from the wall of the *megaspore*.

The male gametes of the genera *Phylloglossum*, *Lycopodium* and *Selaginella* (Lycopodiales) resemble those of the Bryophyta in being motile and biflagellate. All other genera of the Pteridophyta produce multi-flagellate antherozoids.

The Ferns are *homosporous*, bearing one kind of spore only, and the prothallus is normally monoecious; the Horsetails are also homosporous, but usually the prothallus is dioecious. In the *heterosporous* genera *Selaginella* and *Isoëtes* (Lycopodiales), on the other hand, the unisexual gametophytes arise from two types of spores, the male-producing microspores and the female-producing megaspores.

FILICALES

ASEXUAL REPRODUCTION.—Normally the asexual reproductive organs, the sporangia, are borne in groups, the *sori*, on the undersides of the foliage leaves (fronds) of the sporophyte (fig. 51, a). With a few exceptions, each sorus is covered by a flap of tissue, the *indusium*, a specialized outgrowth of the placenta. In some cases, *e.g. Pteridium*, where the sori fringe the pinnule margin, the incurved edge of the pinnule gives the necessary protection.

Occasionally, as in the Hard Fern, *Blechnum spicant*, there is some distinction between the spore-bearing fronds or *sporophylls* and those with a purely vegetative function. In this species the fertile fronds are more upright, have narrower lobes and are almost entirely devoted to the production of the reproductive organs. In the Royal Fern, *Osmunda regalis*, the sori are borne on both sides of the upper pinnae of the frond, while the lower are completely sterile.

The young sporangium, which is the product of a single superficial cell of the placenta, is a stalked, biconvex structure with a wall one cell in thickness, an inner nutritive layer, the *tapetum*, and a central *archesporial cell*. This latter divides to give rise to the sporocytes, each of which, by two further divisions, during which the chromosome number is reduced, produces a tetrad of spores.

The spores germinate to produce a short green filament, which, by division of the apical cell, becomes the flat heart-shaped prothallus. This small thalloid plant, rarely more than one centimetre in diameter, bears on its under surface antheridia and archegonia, the former scattered on the thinner margin and older basal parts, and the latter embedded in

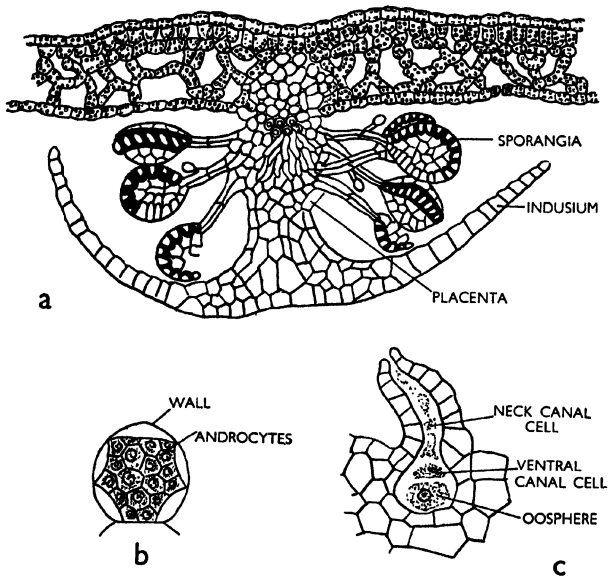

FIG. 51.— a, transverse section through the sorus of the Male Fern (*Nephrodium felix-mas*) showing indusium, placenta and sporangia. b, antheridium of a Fern with wall and androcytes. After Kny, redrawn. c, archegonium of a Fern showing oosphere, ventral canal cell and disorganising neck canal cell.

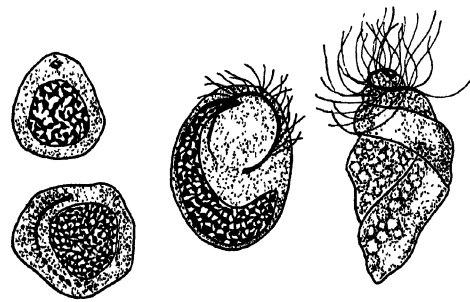

FIG. 52.—Stages in the transformation of the spermatid of Equisetum into the spermatozoid (antherozoid). After Sharp, redrawn.

the thicker central cushion. The less robust prothalli often bear only antheridia.

The antheridium (fig. 51, b), which arises from a single cell, is a comparatively simple structure, consisting of one or two central spermatogenous cells, surrounded by two superimposed ring-shaped cells and covered by a rounded cap cell. By repeated divisions of the central cells, the androcytes arise. In *Marsilia*, two primary spermatogenous cells, by four successive divisions, produce sixteen spermatids. Centrosomes, accompanied by distinct cytoplasmic radiations, develop at the spindle poles during the anaphase of the second of these divisions, divide in the telophase, but subsequently disappear in the cytoplasm. They reappear during the anaphase of the third division and again divide in the telophase when the daughter centrosomes move apart to occupy the poles through the final mitosis. Towards the end of this division each centrosome becomes vacuolate and, in the spermatid, breaks into fragments. During the development of the antherozoids these fragments unite to form a flagella-bearing band which, together with the nucleus, elongates spirally to form the body of the antherozoid.

The archegonium (fig. 51, c) also arises from a single initial cell. Mucilage formed by the breakdown of the neck canal cell (in the Marattiaceae there are two) forces apart the four cells at the apex of the neck, thus allowing the entry of the male gametes. It is believed that these are attracted to the mature archegonia by the presence of malic acid in the exuding mucilage.

After fertilization a thin wall forms round the developing oospore which, after three successive divisions, appears as a group of eight cells arranged as the octants of a sphere. From one half of this structure the stem and first leaf of the sporophyte arise, and from the other the primary root and the absorbing foot.

EQUISETALES

The Horsetails, although an important element in the flora of the Carboniferous period, are now represented on the earth's surface by a single genus of about twenty-five species. Like the ferns, the familiar plant is the leafy sporophyte, the gametophyte being the insignificant prothallus. Although the sporangia are often borne at the apices of ordinary vegetative shoots, there may be, as in *Equisetum arvense*, a distinct difference between the purely vegetative stems and the specialized unbranched fertile shoots, which mature early in the year, and bear, apart from whorls of brown scale leaves, only the reproductive organs.

In all species of *Equisetum* the sporangia are attached in rings, of from five to ten, to the under-surface of stalked peltate scales, the so-called *sporophylls* (spore-bearing leaves). It is probable, however, that these organs are more correctly *sporangiophores*, for a study of the extinct

Equisetales indicates that within this group the true sporophylls have gradually been suppressed.

The sporophylls are grouped together to form characteristic *cones* or *strobili*. At first they are densely crowded, so that, by mutual pressure, the heads assume a hexagonal form. Later, by elongation of the main axis, they move apart and allow the free dispersal of the mature spores

Each sporangium arises from a single superficial cell. All the spore mother cells do not take part in the formation of spores. Some break down and contribute to the nutrition of the rest, from each of which a tetrad of spores with the reduced chromosome number develops. As the spores ripen, the tapetal cells disorganize and their protoplasts coalesce to form the *tapetal plasmodium* which flows between the developing spores and takes part in the formation of their coats. During the development of the spore, the outer layer of the wall splits to form two spirally coiled bands, the *elators*. These appendages become entangled and cause the spores to be shed in clumps, a feature of considerable importance in a genus with dioecious prothalli.

The ripe spores are uninucleate and contain chloroplasts; like most green spores they are very short-lived. If favourable conditions are encountered, they germinate to produce much branched unisexual prothalli, of which those bearing antheridia are usually the smaller. The sex organs closely resemble those of the Fern and need no further description. In the division from which the spermatid of *Equisetum arvense* arises, the centrosome (blepharoplast) is associated with the development of the organs of propulsion (fig. 52). The mature antherozoid is a spirally coiled, multiflagellate structure, the body of which consists of an elongated nucleus, an anterior cytoplasmic band in which lies a chromatic thread formed from the fragmented blepharoplast, and a posterior globule of cytoplasm containing starch and other inclusions.

LYCOPODIALES

In this group are usually included the four genera, *Phylloglossum* (represented by one species found only in Australia), *Lycopodium*, *Selaginella* and *Isoëtes*. The first three form a natural unit with many characters in common, most interesting of which is the possession of biflagellate antherozoids. The genus *Isoëtes*, on the other hand, with its multiflagellate male gametes and highly specialized leaves, differs sufficiently from the other three to throw some doubt on the correctness of including it in the Lycopodiales. In common with *Selaginella*, however, it possesses a small outgrowth of the sporophyll, the *ligule*, not met with elsewhere in the living Lycopodiales but characteristic of many of the extinct members of this group.

Lycopodium.—This is probably one of the oldest living genera of vascular plants and a study of its more primitive species, although out of place here, throws light on one of the greatest problems of plant evolution, *i.e.* how a transition from a leafless dependent sporophyte, as in the Bryophyta, to a leafy independent, vascular sporophyte, as in the Pteridophyta, might have taken place.

The sporophyte in its simplest form (*e.g. Lycopodium pythyoides*) consists of an unbranched leafy stem. Each leaf bears a kidney-shaped sporangium on its upper surface and the whole sporophyte is, in reality,

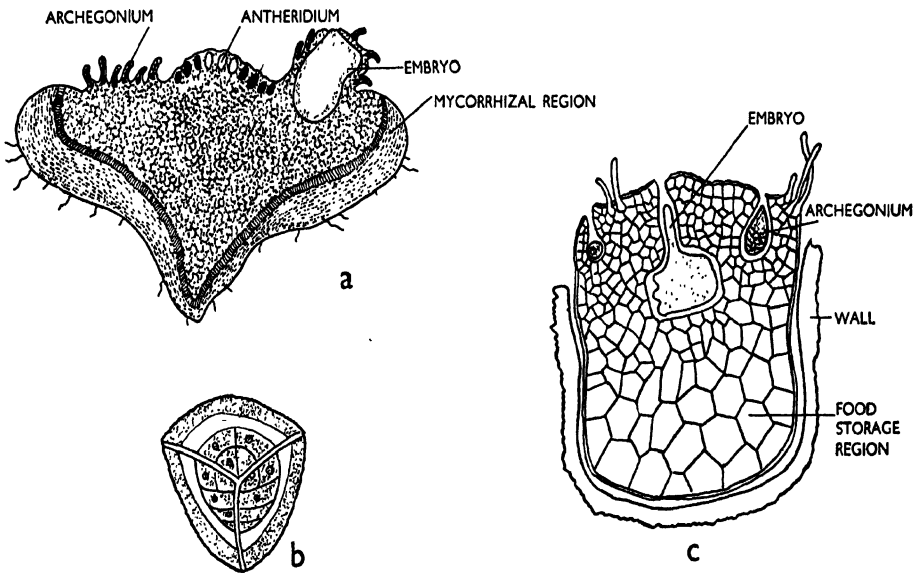

FIG. 53.—a, prothallus of *Lycopodium clavatum* showing antheridia, archegonia, developing embryo and mycorrhizal region. After Bruchmann, redrawn. b, microspore of Selaginella with male gametophyte. After Pfeffer, redrawn. c, female prothallus of Selaginella showing ruptured megaspore wall, archegonia, developing embryo and large-celled food storage region. After Pfeffer, redrawn.

a strobilus of sporophylls. In the more advanced species the lower sporophylls become sterile, so that finally the sporophyte consists of two distinct parts, a sterile lower region bearing only foliage leaves and a compact terminal cone.

The sporangium initially consists of a transverse row of from six to twelve cells, thus differing from the Equisetales in which the sporangium arises from a single superficial cell. Sporogenesis takes place as in other members of the Pteridophyta, each sporocyte giving rise to a quartet of spores with the reduced chromosome number.

The spores germinate to produce the haploid gametophyte (fig. 53, a), a peculiar structure very different from any prothallus yet described. In

its typical form it consists of two regions, an underground tuberous portion, often showing a considerable degree of tissue differentiation and always containing the mycorrhiza of an endophytic fungus, and a lobed aerial crown bearing the sex organs. Two variations of the type occur. In some species the aerial region is completely lacking and the tuberous portion exists as a subterranean saprophyte. In other epiphytic species the gametophyte is totally aerial. Antheridia and archegonia are borne in all cases on the same prothallus.

The antheridium arises from a superficial cell, and gives rise to biflagellate antherozoids. The archegonium, which also develops from a single superficial cell, is embedded when mature in the tissues of the prothallus. Disorganization of the neck canal cells allows the entry of the antherozoids. After fertilization the oospore divides by a transverse wall into an outer suspensor cell and an inner embryonal cell. By elongation, or division, the suspensor cell gives rise to the *suspensor*, an organ which is typical of the Spermaphyta. The embryonal cell divides twice to form a quartet of cells, from one of which the stem develops, from another the first leaf, and, from the remaining pair, the foot.

Selaginella.—Although closely related to the genus *Lycopodium* and possessing many common features, the genus *Selaginella* differs from all the living Lycopodiales except *Isoëtes*, in that it is heterosporous. The sporangia arise from a transverse row of initial cells which occur on the stem just above the point of attachment of the sporophyll, so that they are cauline rather than foliar in origin. When mature, they are shortly stalked and lie in the axil of the sporophyll. The development of the spores up to the formation of the spore mother cells takes place exactly as in *Lycopodium*, but thereafter a fundamental difference occurs. In certain sporangia, usually situated towards the apex of the cone, all the spore mother cells function, and large numbers of small spores, the *microspores*, with the reduced chromosome numbers, are produced. The sporangia in which they occur are known as *microsporangia*. In a small number of sporangia, usually borne towards the base of the cone, only one spore mother cell functions; the others absorb and contribute to the nutrition of that destined to develop further. The functional mother cell divides twice, during which the chromosome number is reduced, and from it arises a tetrad of comparatively large *megaspores* within the containing *megasporangium*. The production of dissimilar spores is known as *heterospory*, in contrast to the *homospory* of other members of the group. The microspores germinate to produce male gametophytes, while female gametophytes arise from the megaspores. The phenomenon of heterospory, rare in the Pteridophyta but universal in the Spermaphyta, is a necessary step in the path which leads to the production of a true seed.

The very simple male gametophyte (fig. 53, b), which never outgrows the spore coat, consists at first of two cells only, the *antheridial cell* and

the *vegetative* or *prothallial* cell. The latter, which represents all that remains of the vegetative tissue of more primitive prothalli, takes no further part in the development of the germinating microspore. The antheridial cell, on the other hand, divides to produce ten or twelve cells —eight sterile *jacket-cells* enclosing two or four (according to species) antherozoid mother cells. From these the spirally coiled, biflagellate antherozoids arise. At maturity the jacket-cells break down and the antherozoids lie freely in the cavity of the microspore, which is carried by wind or gravity to the germinating megaspore.

The megaspore begins to germinate before being shed from the megasporangium, the female prothallus developing at the expense of food stored in the spore. The original nucleus undergoes a series of divisions to produce a large number of free nuclei. Later, separating walls are formed, and, when mature, the female prothallus consists of a mass of small-celled tissue towards the apical (pointed) end of the spore, surmounting a large-celled food storage region (fig. 53, c). A small number of archegonia lie in the apical tissue which eventually protrudes through the ruptured spore wall. After fertilization the embryo develops as in *Lycopodium*, the foot serving to absorb food material from the storage region of the prothallus.

It should be noted that the female prothallus of *Selaginella* is no longer an independent structure but one dependent on the parent plant for its nutrition. This condition is important in connection with the development of the seed habit characteristic of the Spermaphyta, where the process has been carried a stage further by the actual retention of the spore itself within the megasporangium. Even this extreme state of affairs is foreshadowed in *Selaginella*, for in *S. rupestris* often only one megaspore reaches maturity, and this does not leave the megasporangium until after the development of the prothallus and fertilization of the oosphere.

CHAPTER XI

REPRODUCTION IN PLANTS: III. SPERMAPHYTA

IN the Spermaphyta, or seed-plants, heterospory in its most extreme form leads to the production of a single megaspore (*gynospore* or *embryo-sac*), which is retained and develops within the megasporangium (*nucellus of ovule*). After fertilization a specialized structure, the *seed*, is formed.

Two main groups are recognized, the more ancient Gymnosperms, many of which are known only as fossils, and the comparatively modern Angiosperms which form the dominant plant group in the world today. Gymnosperms possess seeds which are naked or exposed, whereas in the Angiosperms the seeds are enclosed in a seed-case or *carpel*, once thought to be a modification of the megasporophyll. To these two groups may be added a third, the extinct Pteridosperms, or Seed-ferns, which, though closely resembling the Ferns in their habit, show affinities with the Gymnosperms in their anatomy and mode of reproduction.

To understand the relationships within the Spermaphyta and to compare the group as a whole with the Pteridophyta, a study of the extinct Gymnosperms should be made. Although this topic cannot be pursued here, it is interesting to note that the two Jurassic genera *Caytonia* and *Gristhorpia* (Caytoniales) possess berry-like fruits consisting of several seeds enclosed in an organ closely resembling the carpel of the Angiosperms. These are the only examples of Pre-Cretaceous plants known to have this typical Angiosperm character.

GYMNOSPERMS

The Gymnosperms include four living families, the Cycadales and Ginkgoales in which the male gametes are motile and multiflagellate and the Coniferales and Gnetales with non-motile male gametes. Of the four groups, the Gnetales most closely resemble the Angiosperms, and it is possible that they represent a modern offshoot of the Coniferales in which an approach is made towards the Angiosperm condition in the structure of the female gametophyte and in the development of the fertilised egg.

To indicate the variety of reproductive behaviour within the Gymnosperms, two genera, *Cycas* (Cycadales) and *Pinus* (Coniferales) will be briefly discussed.

Cycas.—This genus is dioecious, and microsporophylls and megasporophylls, aggregated into distinct male and female cones, are found on separate plants. The microsporangia (*androsporangia*) are borne, often in distinct sori of 2-6 sporangia, on the under surfaces of the thick, spirally arranged, scale-like microsporophylls. They develop as in the Pteridophyta, except that the initial cell or cells are hypodermal and not superficial. Wind- or insect-borne microspores (*androspores* or *pollen grains*) with the reduced chromosome number arise in the usual fashion.

Cycas is unique in that the ovulate strobilus consists of a rosette of sporophylls resembling modified foliage leaves. Each sporophyll bears two or more large megasporangia (*gynosporangia* or *ovules*) on the lower fertile portion. The mature ovule (fig. 54, a) is invested by a thick protective coat, the *integument*, which is fused, except at the extreme apex, with the several-layered parenchymatous wall of the sporangium, the *nucellus*. A passage-way, the *micropyle*, leads through the integument to the *pollen-chamber*, formed by the disorganization of cells at the nucellar tip. A tetrad of megaspores (gynospores), only the lowermost of which survives, arises from the reduction divisions of a single deep-seated spore mother cell. The development of the functional megaspore (embryo-sac) proceeds as in *Selaginella*, and 3-5 archegonia are formed in the small-celled prothallial tissue at the micropylar end. Each develops from a single cell which divides to form a primary neck cell and a central cell. The former divides further to give two neck cells, while the latter enlarges and becomes invested by a layer of nutritive cells. Finally, the division of the nucleus of the central cell results in a ventral nucleus which soon breaks down and an egg-nucleus about which the egg-cell, the largest in the plant kingdom, is organized. In contrast to the archegonia of the Bryophyta and Pteridophyta, no neck canal cells are developed.

While still in the microsporangium, the microspore nucleus divides to form a *vegetative cell* and a second cell which divides to give a *tube cell* and an *antheridial* or *generative cell* (fig. 54, b). At this stage the microspores are shed, and after being carried to the female cone, are drawn into the pollen chamber by the drying up of mucilage which exudes from the micropyle. Here the development of the male gametophyte continues. A sucker-like pollen tube, into which the tube nucleus passes, penetrates the tissue of the nucellus. While still in the body of the microspore the generative cell divides to give a sterile *stalk-cell* and a *body-cell*, the latter dividing again to form two spirally coiled multiflagellate antherozoids (fig. 54, c). Each consists, when mature, of a very large nucleus which undergoes amoeboid movement, a cytoplasmic sheath and a coiled band-like blepharoplast bearing the flagella. When liberated the antherozoids swim to the archegonia through a slimy fluid formed by the disorganization of that part of the nucellar tissue lying between the pollen chamber and the female prothallus. Fertilization is

followed by free nuclear division. Daughter nuclei lying in the lower portion of the egg become separated by cell walls to form the pro-embryo From this, the embryo proper, consisting of two cotyledons, plumule and radicle, develops. An elongated suspensor forces the young embryo into

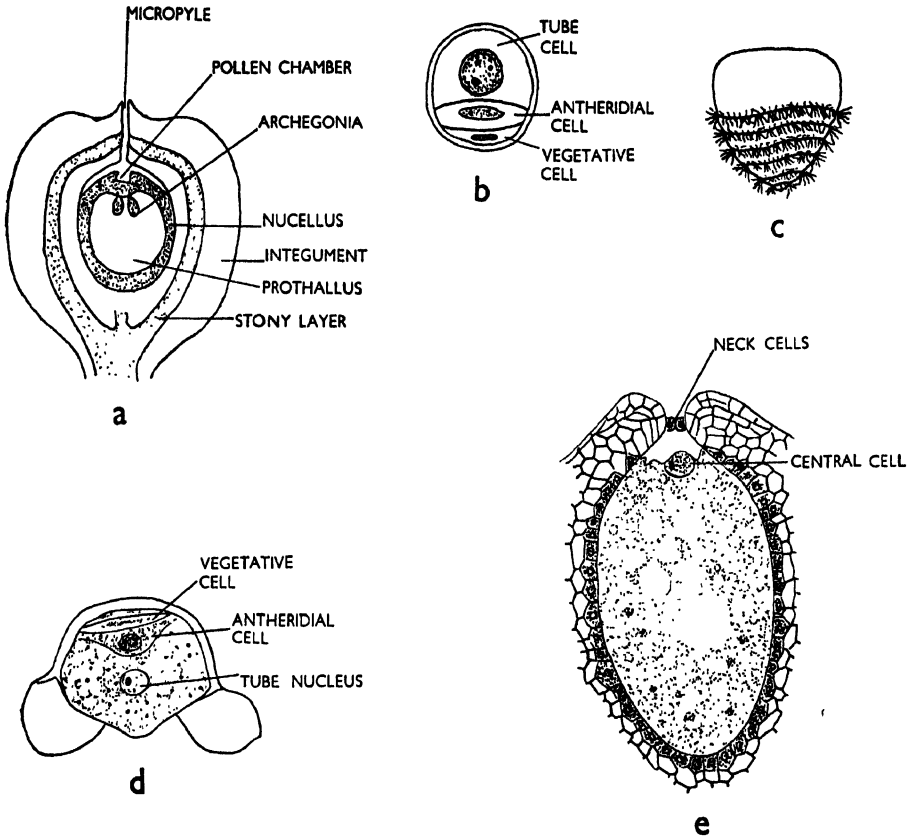

FIG. 54.—a, megasporangium of Cycas (diagrammatic) showing micropyle, integument with stony layer, nucellus, pollen chamber, prothallus and archegonia. After Stopes, redrawn. b, microspore of Cycas with vegetative cell, antheridial cell and tube cell. After Ikeno, redrawn. c, multiflagellate antherozoid of Cycas. d, winged pollen grain (microspore) of Pinus showing vegetative cells, antheridial cell and tube nucleus. e, archegonium of Pinus with two neck cells, and central cell before dividing to give ventral canal cell and oosphere. After Coulter and Chamberlain, redrawn.

the surrounding prothallus, part of which contributes to its nutrition while the rest forms an enveloping *endosperm*. The whole, protected by a thick three-layered coat, the *testa*, formed from the integument, becomes the seed.

Pinus.—Although the reproductive processes of *Pinus*, which is monoecious, are similar in many respects to those of *Cycas*, important

differences occur. Chief of these is the production of non-motile male gametes. These arise in the same way as the motile antherozoids of the Cycadales except that during the development of the gametophyte *two* short-lived vegetative cells are cut off. The pollen grains are winged and wind dispersed (fig. 54, d).

The ovules are borne in pairs on the upper surface of ovuliferous scales each of which is subtended by a smaller bract scale. The micropyles face towards the axis of the cone and the thick integuments are partially fused, on the lower side, to the scales which bear them. The central nucellus of each ovule is united to the integument except in the region of the micropyle. The single haploid megaspore, which arises as in *Cycas*, enlarges and displaces most of the nucellus except for the *nucellar cap*. Formation of the parenchymatous female prothallus usually takes place after pollination, and three archegonia, each with a large oosphere, a small though definite ventral-canal cell and a neck of one or more tiers, are produced at the micropylar end (fig. 54, e).

After pollination the pollen tube commences to penetrate the nucellar tissue, but this growth soon ceases and is not resumed until after an interval of about eleven months. The two male gametes, often unequal in size, then pass to the tip of the elongating pollen tube, which eventually reaches and breaks through the neck of an archegonium, setting free its contents into the egg-cell, or oosphere. The pollen tube of *Pinus* thus acts as a carrier of the male gametes and not solely as an absorbing organ as in *Cycas*. The larger of the two male cells fuses with the egg-nucleus; the smaller degenerates. The fertilized egg-nucleus undergoes two successive divisions and the four daughter nuclei travel to the end of the ovum opposite to the micropyle. Here a four-tiered pro-embryo is formed, from which an elongated suspensor and an embryo with usually five cotyledons develop. The separation of the cells of the lowermost tier often results in the production of several embryos, a condition peculiar to *Pinus*.

The developing embryo gradually absorbs and replaces a large part of the tissue of the female prothallus. That which remains, and in which oil is the chief food reserve, forms the so-called endosperm.

ANGIOSPERMS

The culmination of evolutionary development within the plant kingdom is reached in the Angiosperms. Typically, the megasporangia are completely enclosed within the megasporophyll (carpel), the archegonia of the female prothallus are finally eliminated, and the male gametophyte is reduced to a *tube cell* and a single spermatogenous cell which divides to form two non-motile male gametes.

The cone of the Gymnosperms has given place to the *flower*, a specialized reproductive shoot in which the essential organs are often surrounded by

a characteristic structure, the *perianth*. The flower usually contains both microsporophylls (stamens) and carpels, the former constituting the *androecium* and the latter the *gynoecium*. Such flowers are said to be hermaphrodite. Where stamens and carpels are borne in separate flowers, both the monoecious and dioecious conditions can exist.

The stamens typically consist of two parts, a stalk or *filament*, and a bilobed *anther* bearing the microsporangia (*pollen-sacs*). Divisions of the microsporocytes within the pollen-sacs result in the formation of tetrads of microspores (pollen grains) with the reduced chromosome number

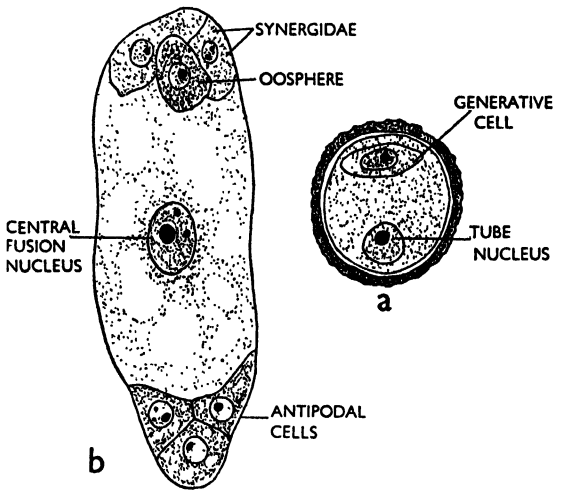

FIG. 55.—a, angiosperm pollen grain (microspore) showing generative cell and tube nucleus. b, female gametophyte of angiosperm showing synergidae, oosphere, antipodal cells and central fusion nucleus.

(fig. 55, a). These are set free by the dehiscence of the anther and are usually wind- or insect-borne.

The carpels generally show three distinct regions: the *ovary* in which the ovules arise; an extension of its tip, the *style*; and a modification of part of the style, the receptive *stigma*. The swollen edges of the individual carpels form cushions of tissue, the *placentae*, upon which the ovules are borne.

Each ovule first appears as a little dome of cells, the nucellus, around which normally two integuments grow. These gradually envelop the ovule until only the micropyle remains. A single hypodermal cell near the apex of the nucellus divides to form a primary wall cell and the megasporocyte. The megasporocyte gives rise to a tetrad of haploid megaspores which lies in the nucellus towards the micropylar end. Of these, the outer three disintegrate, while the remaining spore enlarges

and becomes the embryo-sac. Its nucleus divides, and the two daughter nuclei again divide twice to give a group of four nuclei at each end of the embryo-sac. Three of those at the micropylar end form the egg apparatus, which consists of two auxiliary cells or *synergidae*, and the oosphere, while three at the opposite end become the *antipodal cells*. The two remaining nuclei, the *polar nuclei*, eventually fuse to form the *central fusion nucleus*. This is the usual condition within the embryo-sac at the time of fertilization, where the eight nuclei represent the female prothallus, and the egg apparatus a very reduced archegonium (fig. 55, b).

Although great uniformity in the mode of development of the female gametophyte exists in the Angiosperms, some interesting exceptions occur. In *Lilium*, for example, no cell walls are formed between the nuclei resulting from the division of the nucleus of the megasporocyte, so that only one megaspore with four haploid nuclei is formed. These divide again to give eight nuclei which are distributed within the embryo-sac as described above. Still further reduction occurs in *Plumbagella micrantha* where the four nuclei do not divide again. One becomes the functional female nucleus, two fuse in the centre of the sac, and one disintegrates at its base. The nucleus of the egg is therefore a direct product of the meiotic process, a condition not met with elsewhere in the plant kingdom, and representing the ultimate stage in the reduction of the female gametophyte.

Before being shed, the nucleus of the pollen grain divides to give two nuclei, one of which becomes surrounded by a membrane to form the *generative cell*, while the other, the *tube nucleus*, lies freely in the body of the grain. Germination of the pollen grain takes place upon the stigma, where its development is helped by the secretions of papillae which clothe the receptive surface. A pollen tube, believed to be influenced by the chemotropic stimuli of substances formed in the ovules or ovary wall, grows down the style to the ovary. Into this the tube nucleus, followed later by the generative cell, passes. By way of the micropyle and the nucellar cap, the lengthening pollen tube eventually reaches the embryo-sac. Here its tip ruptures to release two male gametes formed by the division of the generative cell. One gamete fuses with the oosphere, while the second fuses with the polar nuclei or the product of their fusion to give the *endosperm nucleus*. This double fertilization is a phenomenon peculiar to the Angiosperms.

As it has resulted from the fusion of three haploid nuclei, two of maternal origin and one of paternal, the endosperm nucleus is triploid, a fact of importance in the study of the inheritance of endosperm characters. Immediately following the second fusion the endosperm nucleus divides. At first numerous free nuclei are formed, but later cell walls arise between them to give a thin-walled tissue, the endosperm, in which food is stored. The endosperm of Angiosperms, therefore, is not the

true equivalent of that of the Gymnosperms which is haploid gametophytic tissue. The real nature of the Angiosperm endosperm is the subject of some doubt. It can be looked upon as prothallial tissue which has failed to develop until after the stimulus of nuclear fusion, as triploid sporophytic tissue or as a triploid abortive embryo.

Typically, from the fertilized egg a small cell is cut off, which, by a series of transverse divisions, forms a filament of cells, the pro-embryo. This usually becomes differentiated into suspensor and embryo, the latter being thrust deep into the endosperm by the elongation of the suspensor. At first the embryo consists of a single terminal cell, but this divides into an octant by three walls at right angles. From the four terminal cells cotyledons and plumule develop, and from the four basal, hypocotyl and radicle. The enlarging cotyledons may completely absorb the endosperm (non-endospermic seeds), or the endosperm may persist until germination takes place (endospermic seeds). Occasionally, part of the nucellus is not replaced by the endosperm and is recognizable as *perisperm* in the seed.

The modification of the one or two integuments to form the testa completes the development of the seed, a structure eminently suited to the dispersal, protection and subsequent nourishment of the young sporophyte. Finally, the ovary wall becomes the *pericarp* of the Angiosperm fruit, the climax of evolutionary progress within the plant kingdom.

Apomixis in the Angiosperms

A system of reproduction, which, while superficially resembling sexual reproduction, omits one or both of its characteristic features, reduction and gametic fusion, is known as *apomixis*. Various forms of this modification of the sexual process can be recognized, and apomictic species occur in many genera of the Angiosperms. Where the sporophytic generation arises from the female gamete without fertilization, the phenomenon is known as *parthenogenesis*. This can be either haploid or diploid according as to whether or not meiosis has taken place. Haploid parthenogenesis has been reported in various genera (*e.g. Datura*, *Zea*, *Crepis*, *Triticum*), but is of infrequent occurrence. Diploid parthenogenesis, on the other hand, is a regularly occurring phenomenon in many Angiosperms. In most of the forms studied, normal meiosis has been replaced by a single non-reducing division of the embryo-sac mother cell and a diploid embryo-sac has developed from one of the two daughter cells (*e.g. Taraxacum*), but cases do occur where the gametophyte develops directly from the embryo-sac mother cell (*e.g. Antennaria*). In every instance the egg-cell gives rise to the sporophyte without fertilization.

Apogamy occurs when cells of the gametophyte other than the egg-cell, *e.g.* synergidae or antipodal cells, develop into the sporophyte. The

supernumerary embryos observed in *Allium odoratum* are believed to arise in this way. *Aposporγ*, the development of the gametophyte from unspecialized cells of the nucellus or integument, is reported from many genera. In various *Hieracium* species unreduced aposporous gametophytes develop from nucellar cells and may outgrow and replace the normally produced haploid embryo-sacs. The formation of a new sporophytic generation directly from a cell or cells of the nucellus without any intervening gametophytic stage is known as *nucellar embryony*. This has been described in many Angiosperms where it occurs in genera with normal sexual reproduction (*e.g. Citrus*, *Zygopetalum*, etc.) and in those which are parthenogenetic or apogamous (*e.g. Alchemilla*, *Ochna*, etc.).

Gustafsson (1940) in a paper on the interrelation of meiosis and mitosis reviews the various apomictic phenomena encountered in Angiosperms. He uses the term *agamospermy* to cover *diplospory*, apospory and nucellar embryony, where diplospory is the formation of the gametophytic generation from archesporial cells and their descendants and thus includes both *diploparthenogenesis* (diploid parthenogenesis) and *apogamety* (apogamy). The development of the egg-cell in an aposporously produced gametophyte, also, according to Gustafsson, implies parthenogenesis, so that both diplospory and apospory can be followed by parthenogenesis, apogamety or both. He regards nucellar embryony and apospory as closely related phenomena, where the former is a special case of apospory in which the aposporous embryo-sac is reduced to the point of being unicellular. Where nucellar embryos arise, not from a single cell but from groups of cells, nucellar embryony is simply a form of vegetative propagation.

For fuller accounts of apomixis and its possible causes the student should consult the works of Darlington (1937) and Gustafsson (1940).

CHAPTER XII

THE CHROMOSOMES AND HEREDITY

THE rediscovery in 1900 of Mendel's work on heredity, and the recognition that the history of the chromosomes during gametogenesis and fertilization gives a cytological explanation of his work, opened up new fields in nuclear cytology and led to the establishment of the science of *cytogenetics*. The study of the nucleus in relation to heredity has proved to be most fruitful, but as it is undesirable to deal fully with the subject here, the following account is intended to serve as a brief introduction only. For further information, reference should be made to the works listed in connection with the present chapter.

The results of Mendel's experiments with garden peas, although first recorded in 1866, remained unnoticed until rediscovered independently by Correns, De Vries and Tschermak. Mendel dealt with a few sharply defined characters, and his work showed that the characters concerned were transmitted from the parents to the offspring according to certain laws. Considerable knowledge of the chromosomes of the germ-cells was obtained prior to 1900, and between 1900 and 1903 further light was thrown on their behaviour during the meiotic divisions. Sutton in 1902 and 1903, and De Vries in 1903, were the first to give a clear and adequate cytological explanation of Mendel's work.

MENDELIAN HEREDITY

Mendel found that if a pure race of tall peas is crossed with a pure race of dwarf peas all the offspring of the first generation (*first filial generation, or* F_1) are tall. If the tall hybrids are self-pollinated the individuals of the next generation (*second filial generation, or* F_2) are tall and short in the *ratio of* 3 : 1. The dwarfs of the second filial generation when self-fertilized produce dwarfs only, and are, therefore, pure for dwarfness. The tall peas of the F_2 generation contain individuals which are unlike as regards their genetic constitution. One-third is pure for the character of tallness and, if self-fertilized, gives tall individuals only. Two-thirds, if self-pollinated, produce tall and dwarf peas in the ratio of 3 : 1, and, therefore, carry the factors for both tallness and dwarfness. These results can be explained by assuming that the germ-cells of the pure tall

parents carry a factor for tallness and those of the pure dwarf parents a factor for dwarfness (fig. 56). The individuals of the F_1 generation receive the factor for tallness from one parent and the factor for dwarfness from the other parent, but as tallness is *dominant* and prevents the expression of the dwarf character all the individuals of this generation are tall. The young male and female germ-cells of the F_1 peas contain a factor for tallness and a factor for dwarfness, and during the reduction division the two

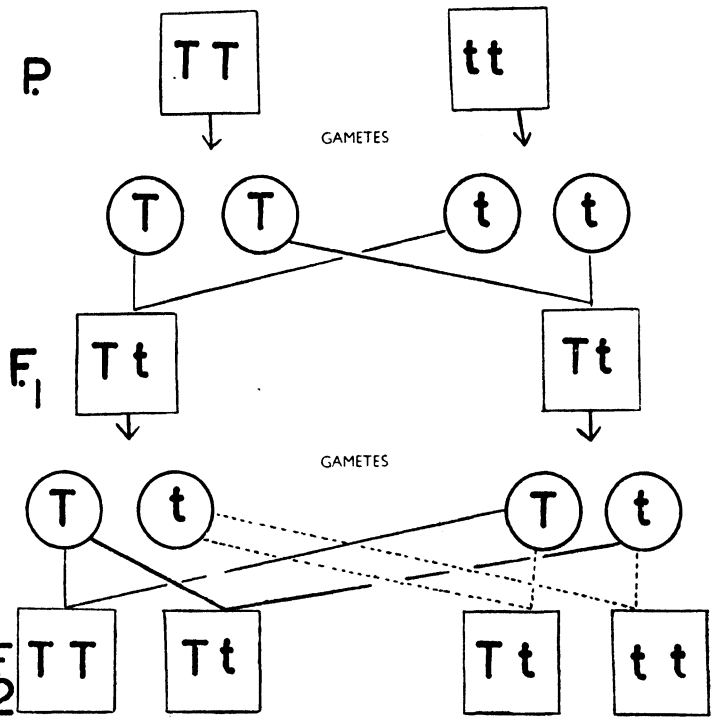

FIG. 56.—Diagram illustrating inheritance with dominance. Individuals are represented by squares and gametes by circles.

factors segregate, so that half the ripe germ-cells contain the factor for tallness and the other half the factor for dwarfness. On self-fertilization the union of the gametes results in offspring of three kinds—25 per cent contain two factors for tallness, 25 per cent contain two factors for dwarfness, and are pure for tallness and dwarfness respectively; 50 per cent carry a factor for tallness and a factor for dwarfness, and on self-fertilization give talls and dwarfs in the ratio of 3 : 1. Breeding experiments which deal with single or *unit characters* in animals yield similar results. The behaviour of the chromosomes during the meiotic divisions offers a cytological explanation of the *segregation of the factors* concerned.

SEGREGATION.—The hereditary characters of an animal or a plant

are determined by minute bodies, the *genes*, which are arranged in *linear series* in the chromosomes. The physical nature and chemical composition of the gene is still unknown and there are many suggestions as to its nature and mode of operation. The gene is the *unit of heredity* and its presence, absence or modification, produces definite effects during the development of the individual. If, in the experiment with tall and short peas, *t* represents the *recessive gene* for dwarfness and *T* the *dominant gene* for tallness, then the pure tall parents possess a pair of chromosomes of which each member carries the dominant gene *T*, and the recessive gene *t* is located in the same pair of chromosomes of the pure dwarf parents (fig. 56). When both members of a pair of chromosomes carry the same gene the organism is said to be *homozygous* as regards that particular gene, and when one member of the pair possesses the dominant gene and the other member the recessive gene the organism is said to be *heterozygous*. Owing to the reduction division, all the gametes of the pure tall parent contain a single chromosome carrying the gene *T*, and all the gametes of the pure dwarf parent possess the gene *t* located in the corresponding chromosome. The F_1 generation have the genetic constitution *Tt*, and produce gametes half of which have a chromosome possessing *T* and the other half a chromosome in which is located *t*. It will be seen (fig. 56) that when mated together this generation produces individuals of three genetic groups, or *genotypes*—homozygous tall (*TT*), heterozygous tall (*Tt*) and homozygous dwarf (*tt*); owing to dominance of the *T* factor, the heterozygotes will be tall. The factors determining two alternative hereditary characters, such as tallness and dwarfness in peas, are known as *allelomorphs*. According to Mendel's first law—*Allelomorphic genes are segregated during maturation into different gametes.*

Breeding experiments show that alternative hereditary characters are segregated during the maturation of the germ-cells, and that when mated together the F_1 generation give a 3 : 1 ratio in respect to these characters. If a *Drosophila* with *normal wings* is mated to one with *vestigial wings*, all the offspring have *normal wings*. *Normal wing* is, therefore, *dominant*, and *vestigial wing* is *recessive*. On mating the individuals of the F_1 generation together, the F_2 flies have *normal* and *vestigial* wings in the proportion of 3 : 1.

The *3 : 1 ratio* refers to a *single pair of allelomorphs* and expresses the *degree of probability* of obtaining the various *genetic types* brought about by the *random combination* of the *gametes*. Consequently, the greater the number of individuals considered the more closely is the exact ratio approached.

The experiments cited above are examples of *inheritance with dominance*, and while this is the most frequent type, it is not the only one. For example, if a black Andalusian fowl is crossed with a splashed white Andalusian, the offspring are blue. The blue Andalusians are hetero-

zygous, and on being mated together produce black, blue, and white individuals in the ratio of 1 : 2 : 1. The black and the white fowl of the F_2 generation are homozygous and breed true, while the blue individuals yield black, blue, and white fowl in the same proportion as the blue Andalusians of the F_1 generation. This is an example of *inheritance without dominance*; the factors are segregated in the same way as those concerned with dominant and recessive characters.

INDEPENDENT ASSORTMENT.—According to Mendel's second law, as modified by later results, *different pairs of allelomorphic genes, when located in different pairs of chromosomes, are assorted independently during maturation into different gametes.* When *two pairs of factors* are involved and *each allelomorphic pair* is located *in different pairs of chromosomes*, the F_2 generation gives a ratio of 9 : 3 : 3 : 1 in respect to these characters. For example, if a *Drosophila* with normal grey body colour and vestigial wings is crossed with a fly possessing long wings and ebony body colour, all the F_1 individuals have long wings and are grey (fig. 57). When the F_1 flies are mated together *four types* emerge in the following proportion—*9 long-winged grey; 3 long-winged ebony; 3 vestigial-winged grey; 1 vestigial-winged ebony.* This shows that grey is dominant to ebony, that the genes for wing length and body colour are *assorted independently*, and are therefore *located in different pairs of chromosomes* When the characters are considered separately, the ratio of 9 : 3 : 3 : 1 breaks down to give 3 long-winged to 1 vestigial-winged, and 3 grey to 1 ebony.

LINKAGE.—Many genes are located in the same chromosome and tend to remain together, so that there are fewer recombinations in the progeny of a cross than would be expected from the random union of the gametes. *Linkage* is between characters whose genes are located in the same chromosome, and, therefore, tend to be inherited together.

CROSSING OVER AND RECOMBINATION.—In a certain percentage of cases, genes which are normally linked are not inherited together, so that a particular character now appears in an individual together with a character the gene for which is known to be located in the other member of a pair of chromosomes. Recombination of linked characters is due to the *exchange of parts* between chromosomes with formation of *chiasmata* which takes place during the prophase of the first meiotic division (p. 45). In *Drosophila*, the characters grey body colour and long wings are dominant to black colour and vestigial wings respectively. If flies having these alternative characters are crossed, the F_1 individuals are all grey and possess long wings. The genes for grey and for long wing are located in a chromosome derived from one of the parents, and the genes for black and for vestigial wings are present in its homologue derived from the other parent. If an F_1 female is mated to a *double recessive* male, four types of flies appear in the next generation—in approximately the following proportion—grey long-winged 41·5 per cent, black vestigial-winged 41·5 per

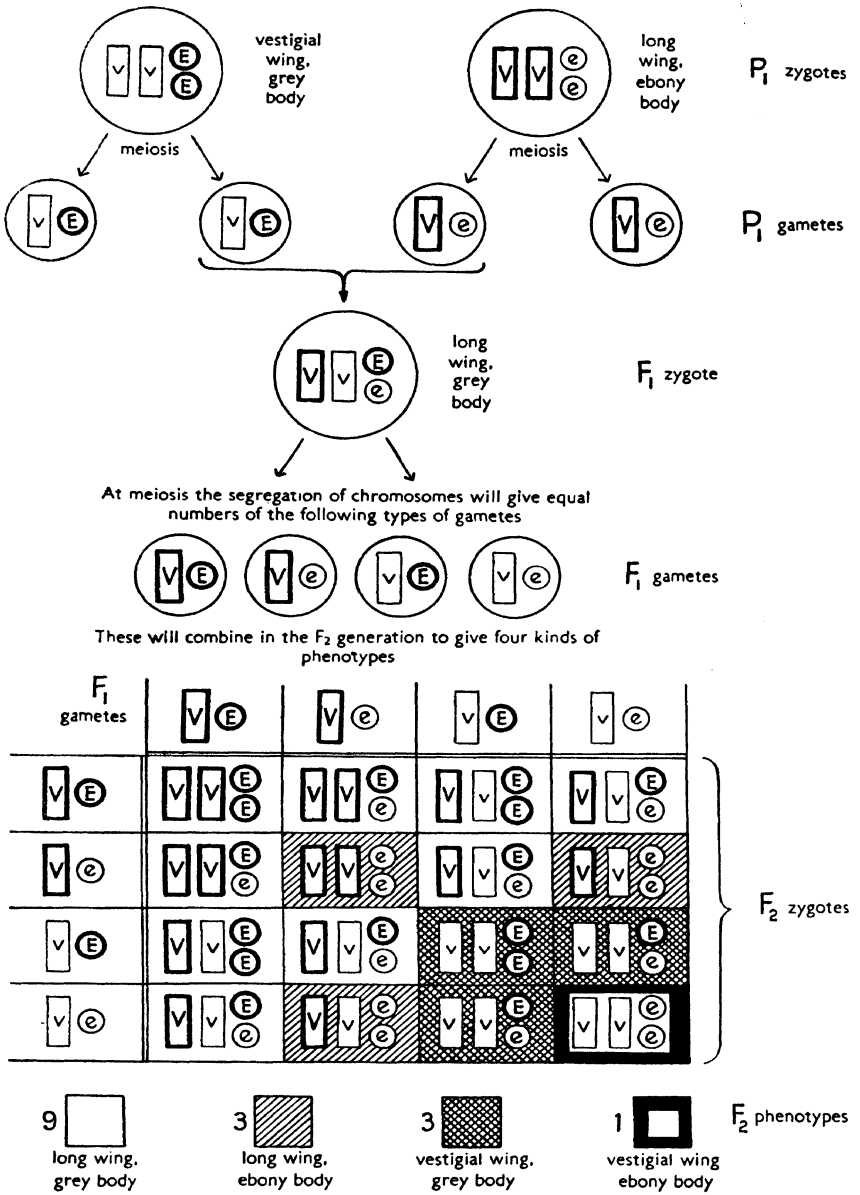

FIG. 57.—Diagram illustrating independent assortment. From Shumway, after Curtis and Guthrie, modified.

cent, grey vestigial-winged 8·5 per cent and black long-winged 8·5 per cent. The characters grey and long-winged and black and vestigial-winged appear in the majority of the offspring and are therefore linked. The new types, grey vestigial-winged and black long-winged, are due to *crossing-over* which takes place between a pair of homologous chromosomes during the maturation of some of the oocytes of the F_1 female. In the male *Drosophila*, crossing-over does not normally take place.

CHROMOSOME MAPS.—Different pairs of allelomorphic genes give different percentages of crossing-over, the frequency varying with the distance between the genes involved. Chromosome maps showing the serial order of the genes have been constructed, taking 1 per cent of crossing-over as one unit on the map between the genes concerned. Such a map does not show the precise positions of the genes on the chromosome. Information regarding the location of genes may be obtained by the study of *deletions*. The loss of small parts of a chromosome may be induced by irradiation, and by noting the characters affected, the genes situated in the deleted portion can be determined. Portions of some chromosomes contain no known genes. Such *genetically inert* regions are often situated near the centromeres, but may also be found in other positions.

THE SEX CHROMOSOMES

The male of certain organisms possesses a pair of chromosomes whose members differ visibly from each other, while the nuclei of the female contain a pair of chromosomes which resemble one member of the *heteromorphic* pair present in the male. These chromosomes are known as the *sex chromosomes*, and all the other chromosomes are called *autosomes*. The female has two sex chromosomes, known as X chromosomes, and the male possesses two sex chromosomes, one of which is called an X chromosome and the other a Y chromosome. The formula for the female is, therefore, XX, and for the male XY. The Y is usually smaller than the X, but in some cases it is larger, and in other cases the two chromosomes are of equal size. In this example the female is said to be the *homogametic sex* and the male the *heterogametic sex*. All the ripe ova contain a single X chromosome, while two types of spermatozoa are produced in equal numbers, one type has an X and the other a Y (fig. 58). Random union of the gametes results in females (XX) and males (XY) in approximately equal numbers

In some organisms, chiefly birds and some of the Lepidoptera, the female is heterogametic and possesses an X and a Y chromosome; the formula for the female is XY and for the male XX. In some cases the Y is absent, so that one sex has a single X chromosome and the other a pair of X chromosomes (fig. 59). The XX–XY type of sex chromosome mechanism is, however, of more frequent occurrence and is found in many animals and plants.

In Bryophytes the gametophytes are haploid; the female has a single large X chromosome, and the male a small Y. The zygote, therefore, has an X and a Y, and develops into the asexual sporophyte. Meiosis results in four haploid spores, two of which possess an X and give female gametophytes, and two have a Y and develop into male gametophytes.

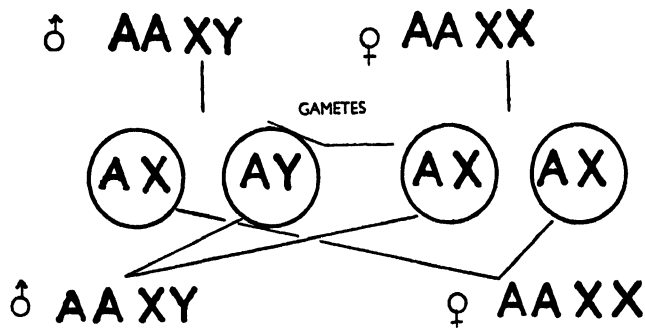

FIG. 58.—Diagram illustrating the distribution of the sex chromosomes during maturation and fertilization. XX-XY type. In this example the male is the heterogametic sex.

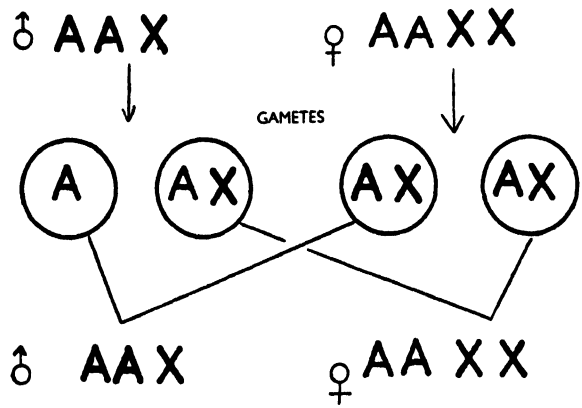

FIG. 59.—Diagram illustrating the distribution of the sex chromosomes during maturation and fertilization. XX-XO type. In this example the male is the heterogametic sex.

Reference has already been made to the behaviour of the chromosomes in male and female-producing eggs of diploid parthenogenetic females (p. 71). In diploid parthenogenesis there is no reduction in chromosome number and, except in male-producing eggs, the offspring have the same chromosome constitution as the parent and are females. There is little knowledge of the sex chromosomes of animals which reproduce by haploid parthenogenesis. The origin of male haploidy is discussed by Whiting (1945).

Sex chromosomes have not been identified in some organisms. They are not the only factors responsible for the determination of sex, and there is evidence that the sex of some animals is influenced by environmental conditions. In certain animals sex reversal is known to occur in nature and it has been induced experimentally in, for example, Amphibia. In these cases some other factor overrides the influence of the sex chromosomes, so that an animal possessing the sex chromosome constitution of one sex is transformed into the other sex. The sex ratio of the offspring of frogs which have undergone sex reversal shows that the parents maintain the chromosome composition of the original sex, and that the sex chromosomes are important factors in the determination of sex. It would appear that the sex chromosomes are one of several factors concerned with sex-determination, and that they act as a differential mechanism which normally results in the development of male and of female organisms.

SEX-LINKED INHERITANCE.—As the sex chromosomes of certain animals and dioecious plants carry genes for non-sexual characters, it follows that these genes are linked with those determining sex. If the gene concerned is located in an X chromosome the corresponding character will appear in both males and females, but if recessive may not be expressed in the homogametic sex. For example, in man, in which the male is heterogametic, the recessive X-borne gene for colour-blindness is transmitted by a homozygous female to all her offspring. If her husband has normal colour vision the daughters will be heterozygotes and therefore normal, but all her sons will be colour-blind. Other combinations, of course, give different results. Factors present in the Y chromosomes are always associated with the heterogametic sex; few genes, however, have been identified in Y chromosomes.

STRUCTURAL REARRANGEMENTS OF THE CHROMOSOMES

Structural rearrangements of the chromosomes have been induced experimentally, but spontaneous rearrangements are not common. Some rearrangements have already been mentioned and have been used in the study of the chromosomes as the bearers of genes.

INVERSION.—Sometimes the position of a portion of a chromosome becomes reversed in comparison with the normal sequence. An *inversion* is brought about by a chromosome thread breaking at two points and the broken ends rejoining in such a way that the position of part of the chromosome is reversed. Inversion seldom occurs near the ends of a chromosome, but is usually intercalary. During meiotic pairing the inverted region pairs with the homologous region of the other chromosome so that a loop is formed (fig. 24).

DELETION.—A *deletion* is brought about in the same way as an inversion, but in this case the part between the breaks is lost and the broken ends rejoin. The chromosome affected pairs with the homologous parts of the other member of the pair, and the unpaired region of the latter forms an unpaired loop. Deletions are usually intercalary (fig. 60).

TRANSLOCATION.—Sometimes a portion of a chromosome is transferred to another chromosome which may not be homologous.

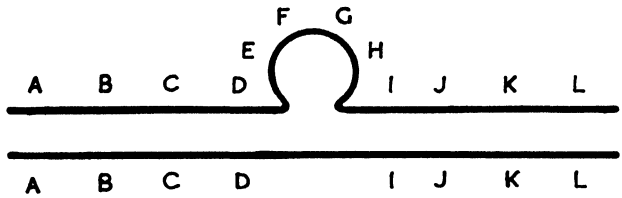

FIG. 60.—Diagram illustrating deletion.

Translocations may or may not involve a reciprocal exchange of parts. One of the resulting chromosomes may be without a centromere, while the other possesses two; consequently they do not behave normally during mitosis. If each chromosome is provided with a centromere they will both behave normally. Reciprocal translocations have been found in a number of animals and plants in nature.

DUPLICATION.—Certain regions of a chromosome are sometimes present more than once. The regions may be situated next to one another or may be separated, they may be inverted or occupy their normal positions in the chromosome.

CHAPTER XIII

THE CHROMOSOMES AND EVOLUTION

WE have seen that the *somatic nuclei* of a *diploid* organism contain two *autosomes* of each kind—the *homologous chromosomes* (p. 34)—but organisms are sometimes found in nature with nuclei which possess more than two haploid sets of chromosomes. Nuclei possessing more than the diploid number of chromosomes are known as *polyploid* nuclei (p. 37). Organisms containing three of each kind of chromosome in their nuclei are called *triploids*, those with four of each kind are called *tetraploids*, and much higher numbers than these are known to occur. Polyploidy is due to multiplication of one or both of the haploid sets of chromosomes and arises through irregularities of mitosis or of meiosis. If the multiplication takes place in a hybrid between two species the condition is known as *allopolyploidy*, and in this case the chromosomes are derived from different species and the haploid sets are not all identical. If polyploidy occurs in an individual which is not a hybrid, the nuclei are said to be *autopolyploid*. Sometimes not the whole haploid set is multiplied, but only one or more chromosomes. The resulting condition is called *polysomy*. A polyploid organism gives germ-cells in which each chromosome may be diploid, triploid, tetraploid or a higher multiple, according to the degree of polyploidy of the organism.

Polyploidy can be induced experimentally by treatment with *abnormal temperatures* and with *colchicine*. It sometimes produces morphological or physiological changes in plants which are of practical importance. Colchicine acts by preventing the formation of the mitotic spindle, so that nuclear division does not take place and a tetraploid nucleus is formed. If a shoot results from a tetraploid cell, its gametes will have chromosomes ranging from haploid to triploid. Fertilization between two fully diploid gametes, or between a fully triploid and a fully haploid gamete, gives tetraploids, and the union of a fully diploid with a fully haploid gamete will produce a triploid organism.

Tetraploid plants are frequently found in nature. They arise as the result of an abnormal somatic mitosis, or an abnormal reduction division which gives diploid gametes. If tetraploidy is established in a very young embryo, all the cells of the resulting plant will contain two diploid sets of chromosomes, but if it occurs at a later stage a plant will be produced with one or more tetraploid parts.

Tetraploids often possess characters which differ from those of the diploid plant of the same species. Frequently their cells and nuclei are larger, they are darker in colour, and they produce bigger flowers and seeds. The fruit of tetraploid tomato plants have a higher vitamin C content, and the yellow kernels of maize a higher vitamin A content than those of diploids. The formation of quadrivalent groups at the first meiotic division of autotetraploid plants leads to irregularities in the distribution of the chromosomes and consequently to gametes with unbalanced chromosome complements, and to sterility. Tetraploidy in hybrids, however, often gives increased fertility, and when induced experimentally in sterile hybrids sometimes results in a higher rate of fertility.

In some triploids fertility is high but in others it is low. Generally the offspring have a low survival value, and consequently are not usually found in nature among groups which reproduce sexually. Triploids of some domestic plants are propagated by vegetative methods.

The chromosome number of the different species of certain genera of plants are all *multiples* of a *basic number*. In some genera the numerical relationship between the chromosomes of the various species is less striking, and in others no such relationship exists. Further, the chromosome complements of the genera of some families bear a numerical correlation with one another, while in other families the chromosome number of all the genera is the same. In some cases, therefore, the study of the chromosome number of the species of a genus, or of related genera, yields evidence of genetic and evolutionary relationships.

It is believed that changes in the characteristics of an organism which lead to species formation originate as changes in the chromosomes. The appearance of a new character is sometimes due to alterations in the number or structure of the chromosomes, but is often brought about by *gene mutations*.

The view that new species have arisen through alterations in the chromosomes is supported by experimental evidence which shows that plants with new characters have appeared as a result of polyploidy, and that structural rearrangements of the chromosomes and gene mutations may be induced in *Drosophila*, and in some other animals, by treatment with X-rays and other agents.* That structural rearrangements have taken place in nature is indicated by some related species which all possess the same number of chromosomes in their nuclei, but the chromosomes of the various species differ in length and in the position of the spindle attachments.

Polyploidy appears to be relatively rare among animals except in highly specialized and degenerating cells. Fankhauser (1945) reviewed

* Mutations and structural rearrangements of the chromosomes have been obtained by exposing *Drosophila melanogaster* to mustard gas vapour (Auerbach, C., Robson, J. M., and Carr, J. G., 1947. "The Chemical Production of Mutations", *Science*, 105, p. 243.

the literature of the subject and described polyploid salamanders and newts which arose spontaneously in the laboratory and also under experimental conditions. He stated that the frequency of spontaneous changes among salamanders appears to be higher than in most diploid plants, and that the percentage of polyploid individuals produced by experimental treatment is considerably greater than that obtained in plants by similar means. Polyploid nuclei are common in degenerating and in highly specialized cells, such as connective tissue-cells, and are so widespread in adult insects as to be considered the rule rather than the exception. In these tissues it is brought about through special types of mitosis occurring as a mechanism of tissue differentiation.

In some groups of animals the chromosome number is fairly constant. In others it varies from species to species, and these variations appear to be brought about by means other than polyploidy.

As a chromosome is usually provided with a single centromere, and as the latter must be present in order that a chromosome may become attached to the spindle, it follows that an increase in the number of chromosomes in a nucleus cannot normally be due to fragmentation. That broken parts of a chromosome devoid of a centromere do not become attached to the spindle is illustrated by the behaviour of the ends of the large chromosomes of *Ascaris equorum* (fig. 42). The middle region of the large chromosomes is provided with several centromeres, and in the germ-cells and cells of the germ-track these act together. In the blastomeres which give rise to somatic tissues only, the central region breaks up to form a number of small chromosomes, each provided with a centromere. New centromeres arise through the division of pre-existing ones, and it is possible that the very small chromosomes of *Drosophila*, and of some other animals, may have arisen through the duplication of the region of the spindle attachment of large chromosomes. In *Drosophila* the part of the chromosome adjacent to the centromere is *genetically inert*, and consequently the duplication of this region would not reduce the capacity of the organism to develop, as is probably the case when a whole chromosome is duplicated. It is known that only small regions of the salivary gland chromosomes are duplicated, and it is suggested that the small chromosomes formed by duplication may be converted into large ones by translocations from other chromosomes. In some species of animals one or more chromosomes, while absent in some individuals are present in others without visibly affecting the appearance of the organism. It is probable that such supernumerary chromosomes have arisen from a normal chromosome by deletion and also by translocation from several chromosomes. White (1945) states that " the formation of supernumeraries is probably the chief method whereby chromosome numbers become increased in the course of evolution ".

It is possible that some V-shaped chromosomes may have two centromeres situated close together, and that a break may take place in the

region between the attachments so that two rod-shaped chromosomes are formed. It is also possible that two rod-shaped chromosomes, with sub-terminal attachment, may join together to form a V-shaped chromosome possessing two centromeres. Most species of the Acrididae possess eleven pairs of rod-shaped autosomes with sub-terminal attachment. In certain genera there are eight pairs, five of which are rod-shaped, and three are V-shaped with median or sub-median attachment. Each V is formed by two of the originally separate rods fusing together, and it is probable that the V-shaped chromosomes so constituted possess two centromeres.

The chromosomes of related species, and of races, frequently differ in shape and size, and their study suggests that structural alterations have been brought about by translocations, inversions and deletions (pp. 117-118). For example, the number of chromosomes in *Drosophila melanogaster* and in *Drosophila simulans* is the same, and their relative lengths are practically identical, but it has been established that one-third of the total length of one of the chromosomes of *D. simulans* is inverted. In the salivary glands of the hybrid between the two species this chromosome shows an inversion loop, and there are slight differences in the sequence of the bands of other chromosomes. The hybrids between different geographical races of *Drosophila pseudo-obscura* have been examined, and their chromosomes found to possess different gene arrangements which are due to inversions. The study of the salivary gland chromosomes of hybrids between certain species of *Drosophila* shows that large regions do not pair and, therefore, are not homologous. In hybrids of *D. pseudo-obscura* and *D. miranda* pairing occurs between short homologous regions, but several inverted regions and some translocations are present. The amount of structural change which the chromosomes have undergone is not always an indication of the degree of relationship; for example, the chromosomes of the various races of *D. pseudo-obscura* have greater differences than exist between those of *D. melanogaster* and *D. simulans*.

The examples cited above are sufficient to indicate that quantitative and qualitative changes in the chromosomes have led to the appearance of new characters and to the formation of new varieties and species, and are, therefore, concerned with the process of evolution. In plants, new species have also arisen through polyploidy, but in animals structural rearrangements of the chromosomes and gene mutations are apparently the most important factors.

Species usually differ in many genes, and as mutations affect a single gene at a time, it is probable that new species usually arise as the result of several mutations. Each mutation affects, to a greater or less degree, a single character, or several characters, and further mutations may result in the appearance of an organism which differs sufficiently from the parent stock as to constitute a separate species. As the effect of a gene is determined not only by the nature of the gene itself but also by the genes located

in the adjacent regions of the chromosome, it is probable that structural rearrangements may alter the functions of several genes.

Most, if not all, genes act by controlling specific biochemical steps in the development of an organism (Pontecorvo, 1946). It is not known, however, if the genes act continuously throughout the cell cycle or only at a certain stage of the cycle (*e.g.* the interphase). Each gene exerts a determining influence at a definite stage in the development of an organism. This stage may be extremely short or may extend over the greater part of the developmental period.

While it is believed that evolutionary changes originate in the chromosomes, it is not known with certainty what initiates the alterations within the chromosomes. It is clear that irregularities of mitosis may result in polyploidy, but the mechanism of structural alterations and gene mutation is more difficult to explain. It is known that some genes mutate more frequently than others and that treatment with abnormal temperatures, with ionizing radiations, and with certain chemicals will increase the rate of mutation and structural alterations in the chromosomes. The genes which mutate under experimental treatment are those which do so spontaneously. The agents which in nature cause mutations, or increase their rate, are, however, unknown, and it has been calculated that the radiation present in nature would account for less than 1 per cent of spontaneous mutations.

Many mutations are lethal, and organisms possessing them do not survive. When a non-lethal mutation arises it must be of such a nature that the organism, if it is to survive, is adapted to its environment; there is evidence that a particular environment may be favourable, or unfavourable, for the expression of a particular genotype. If a new variety, or species, is to become established it must be isolated geographically, or prevented in some other way from breeding with the parent stock or related groups; otherwise intermediate types might result and prevent specific distinctness. It is evident, therefore, that while new characters arise through changes in the chromosomes, other factors, which may be roughly classified as chance and natural selection, are later concerned with the establishment of these characters.

CHAPTER XIV

THE CYTOPLASM AND HEREDITY

THE part played by the cytoplasm in development and heredity has been for long a subject of controversy. Some of the earlier workers considered that the cytoplasm had a determining influence on development. Boverie, for example, thought that the form and rate of cleavage and certain general characters of the embryo are determined by the nature of the cytoplasm of the egg, and that the characters of the individual and of the species are determined by the nucleus. According to this view cytoplasmic factors are active during the very early stages of development, and the later stages are under the control of the nucleus. Later, Boverie stated that the cytoplasmic characters are probably originally formed under the influence of the nucleus. Other observers contributed to the controversy, but with the growth of knowledge of the chromosomes and the recognition of their importance as bearers of genes, attention became more and more focused on the study of the nucleus in relation to heredity. In recent years, evidence has been cited in support of the view that determinants are present in the cytoplasm; it is with this more recent work that we are mainly concerned in the present chapter.

In Angiosperms the cytoplasm is mainly maternal in origin. In reciprocal crosses between two species the nuclei of the resulting hybrids are alike, but in some cases their cytoplasm shows differences depending upon which species is the maternal parent. In reciprocal crosses between two species of *Epilobium* certain characters of the offspring always resemble those of the female parent. There is also evidence that chlorophyll deficiency in some plants is transmitted through the maternal cytoplasm, and cytoplasmic inheritance of other characters is claimed for certain plants.

East (1934, (a) and (b)) reviewed much of the evidence for and against cytoplasmic inheritance in plants and animals. He concluded that the early stages of cleavage may be controlled by the nucleus of the female, that there is no evidence to show conclusively that hereditary factors, independent of the nucleus, exist in the cytoplasm, and that nuclear genes are the determining factors. Since the publication of East's conclusions further work has been carried out on plants and animals, but chiefly on plants. Hamburger (1936) obtained reciprocal crosses between certain

species of newts, and selected for study some clearly defined characters. He found that up to the time when the fore-limb buds appear the F_1 hybrids are, with the possible exception of the optic vesicles and the optic cups, of the maternal type. Hamburger concluded that maternal factors control the fundamental characters of early development, and that the time at which the factors introduced by the sperm operate is different for different characteristics. He claimed that the zygote nucleus never gains exclusive control over the growth process. Harvey (1942) obtained hybrids between certain species of Echinoderms, and stated that the early inheritance is maternal and cytoplasmic. Later, Moore (1943) claimed that the sea-urchin hybrids on which he worked are intermediate in type between the two parents.

Most of the evidence of cytoplasmic inheritance is derived from breeding experiments with plants and is concerned almost exclusively with the transmission of maternal characters. In animals much of the evidence is less conclusive and relates mainly to characters of the early embryo or larva which are thought to be maternal in origin. Darlington (1944) regards the components of a cell as parts of a complicated system which continually react with, and mutually influence, one another. His observations will stimulate further advances in the study of development and heredity.

According to Darlington there are three systems or levels of determinants. (1) *The Nuclear System* is the highest level and is "that which is most accurately and equally distributed at the division of the cell and most equally transmitted by the two parents in sexual reproduction. It is responsible for the Mendelian heredity of genes; it determines the widest range of hereditary variation; and its equilibrium is mechanical. Its transmission (with odd exceptions) is not influenced in any regular way by external developmental conditions. It therefore predominates in the government of heredity as well as in the government of the cell." (2) *The Corpuscular System* is recognizable only in green plants. It is liable to be unequal in distribution at cell division, and as it is largely maternal in transmission, it is unequal in inheritance. "Its equilibrium is best described as physiological." Darlington reviewed certain work on plants and stated that observations on reciprocal crosses between two species of *Oenethera* show that the colour of the plastids is determined by the joint reaction of determinants, or *plastogenes*, and the nucleus. The nuclei and the plastogenes are "mutually adapted in each species to the production of chlorophyll, and this adaptation is upset in the hybrid". There is evidence that in delayed and irreversible changes, such as are recorded for the plastids of barley and rice, plastogene mutation is controlled by the nucleus. (3) *The Cytoplasmic* or *Molecular System* is "not associated with any visible bodies in the cell and hitherto supposed to be purely maternal in transmission". Its equilibrium is chemical. Determinants,

or *plasmagenes*, which are protein in nature and suppressive in action, are present in the cytoplasm.

Darlington observes that *viruses* have similar properties to plasmagenes. They are both formed in the cytoplasm and depend on *ribose nucleic acid* for their reproduction, while the nuclear genes arise in the nucleus and depend upon *desoxyribose nucleic acid*. They are both conditionally self-perpetuating, and in heredity and development depend upon the interaction of the nucleus and the cytoplasm. They do not differ in origin or action, but in the method of transmission—the viruses are infective, and the plasmagenes are inherited. Both plasmagenes and viruses are continually arising and evolve " as their conditions change and partly by direct action of these conditions ". Both are subject to nuclear and environmental control.

Darlington suggests that in addition to the nuclear genes and the plasmagenes other proteins are formed by the nucleus, and these, with limited powers of self-perpetuation, are responsible for maternal inheritance. Their capacity for becoming part of heredity, or of becoming infective, depends on suitable nuclear and cytoplasmic conditions.

According to the conception of heredity outlined above, extra-nuclear factors are responsible for the determination of certain characters, but the nucleus plays the dominant part in inheritance. The various hereditary determinants do not act independently; plasmagenes are subject to nuclear and environmental control which influences their action and mutation, and in green plants there is interaction between plastogene and plasmagene. This theory does not seek to disprove the established importance of the chromosomes in inheritance, but by taking a view which embraces contributions from both nucleus and cytoplasm, and the relationship between nuclear and cytoplasmic factors, serves to bring together previous conceptions in a manner which may result in further advances in our knowledge of heredity.

Since the publication of Darlington's paper Lindegren (1945) has given an account of experiments on yeasts and has drawn some interesting conclusions. Lindegren believes that the ability of yeasts to adapt themselves to specific substrates is due to a *cytoplasmic mechanism*, and that, while the *adaptive enzymes* are initiated by nuclear genes, adaptation takes place only through the interaction of the cytoplasm with the substrate He found that an adaptive enzyme, or *cytogene*, could be transmitted through the cytoplasm and maintained in cells lacking the appropriate nuclear gene. Lindegren points out that, as the cytogene is initiated by a nuclear gene and is dependent on the substrate, it differs from Darlington's plasmagene, but might be transformed into a plasmagene by a metabolic process which synthesizes the appropriate molecules. According to this view a cytogene is initiated by a nuclear gene transmitted through the cytoplasm, where it may be maintained in the absence of the nuclear gene,

and may possibly evolve into a plasmagene. Pontecorvo (1946) suggests " that the control by genes of elementary biochemical steps takes place through enzymes ", and that most enzymes are primary gene products.

In the works on cytoplasmic inheritance little mention is made of the possible contribution of the spermatozoon. With reference to Darlington's statement that the molecular system of heredity has been " hitherto supposed to be purely maternal in transmssion ", Haldane (1944) stated that " there is at least one case to the contrary in animal genetics " illustrated by the experiments of l'Héritier and Teissier and of Kalmus. In a short note Kalmus (1943) pointed out that l'Héritier and Teissier found that in *Drosophila melanogaster* the character of *susceptibility* to carbon dioxide was transmitted to all the progeny of a susceptible female, but only to some of the progeny of a susceptible male. Some of the male's susceptible daughters, but not his sons, may transmit the character to their offspring. Kalmus stated that susceptibility is not fixed to any of the chromosomes and cannot be regarded as a Mendelian character. He succeeded in crossing individuals of l'Héritier and Teissier's stock of *D. melanogaster* with individuals of *D. simulans*, and stated that susceptibility is " as a rule transmitted to all the interspecific hybrids by the mother, but only to part of the hybrid offspring by the father ". Kalmus concluded that the results of his experiments suggest, but do not prove, that the progeny of the cross between *D. melanogaster* male and *D. simulans* female contain resistant and susceptible flies in varying proportions. The experiments of l'Héritier and Teissier and of Kalmus strongly suggest that cytoplasmic factors are transmitted by the sperm ; it appears, however, to be the only clear case so far recorded in animals.

It has been demonstrated that the sperm middle-piece is carried into the egg of at least some animals (pp. 69-71), but there is no evidence in support of the view of certain early workers that the mitochondria are concerned with the determination of hereditary characters, and Darlington believes that the plasmagenes are not associated with any visible structures in the cell. As the sperm contains a limited quantity of cytoplasm, it is reasonable to suppose that plasmagenes or cytogenes, if such exist, are transmitted by the male as well as by the female. If this view be correct, then the nucleus is not the only part of the sperm which influences development and heredity, and its cytoplasm has an importance which is not generally recognized. Quite apart from the question of hereditary factors, there is evidence that the sperm contributes certain cytoplasmic bodies to the embryo.

CHAPTER XV

THE MORPHOLOGY AND COMPOSITION OF THE GOLGI MATERIAL AND MITOCHONDRIA

REFERENCE has already been made to the general character of the mitochondria (pp. 19-20) and of the Golgi material (p. 20), and their behaviour during oogenesis (p. 51 and pp. 52-53), spermatogenesis (pp. 56-63), maturation and fertilization (pp. 67-71) has been described. We have seen that the Golgi elements, and possibly the mitochondria, are concerned with the elaboration of yolk, that the Golgi substance takes part in the formation of the acrosome, and that both Golgi material and mitochondria are included in the ripe sperm. We have still to examine certain features of their structure and composition, their function in gland cells, and their behaviour in relation to other aspects of cell activity.

THE GOLGI MATERIAL

Golgi substance has been identified in a wide variety of animal tissues (figs. 61 and 62), and is preserved by osmium tetroxide and silver nitrate techniques. It is provided with the power of growth and multiplication, and is, therefore, a living component of the cell. Golgi material is frequently described as forming a *reticulum* or *fibrous network* lying at one side of the nucleus. Many cytologists, however, believe that it seldom, if ever, forms a true network, but consists of a number of *separate elements* which may lie close together. It is possible that in neurones, and perhaps in some other types of cell, or in certain phases in the history of some cells, it may form a net-like structure. It is probable that it is not a structure with permanent form, but that it assumes different forms which are correlated with the phases of activity, or stages in the life, of the cell. The Golgi material of the eggs of certain animals, it is claimed, is in the form of *vesicular bodies* made up of osmiophilic material surrounding a clear area. Pollister (1939) believes that the Golgi material of the tissues of larval and adult Amphibia is *lamellar* in structure. Simpson (1941) examined vertebrate tissues prepared by the freezing-drying method and stated that the Golgi material is present as a *coiled canal*, the contents of which cannot be blackened by osmium tetroxide. He studied cells subjected

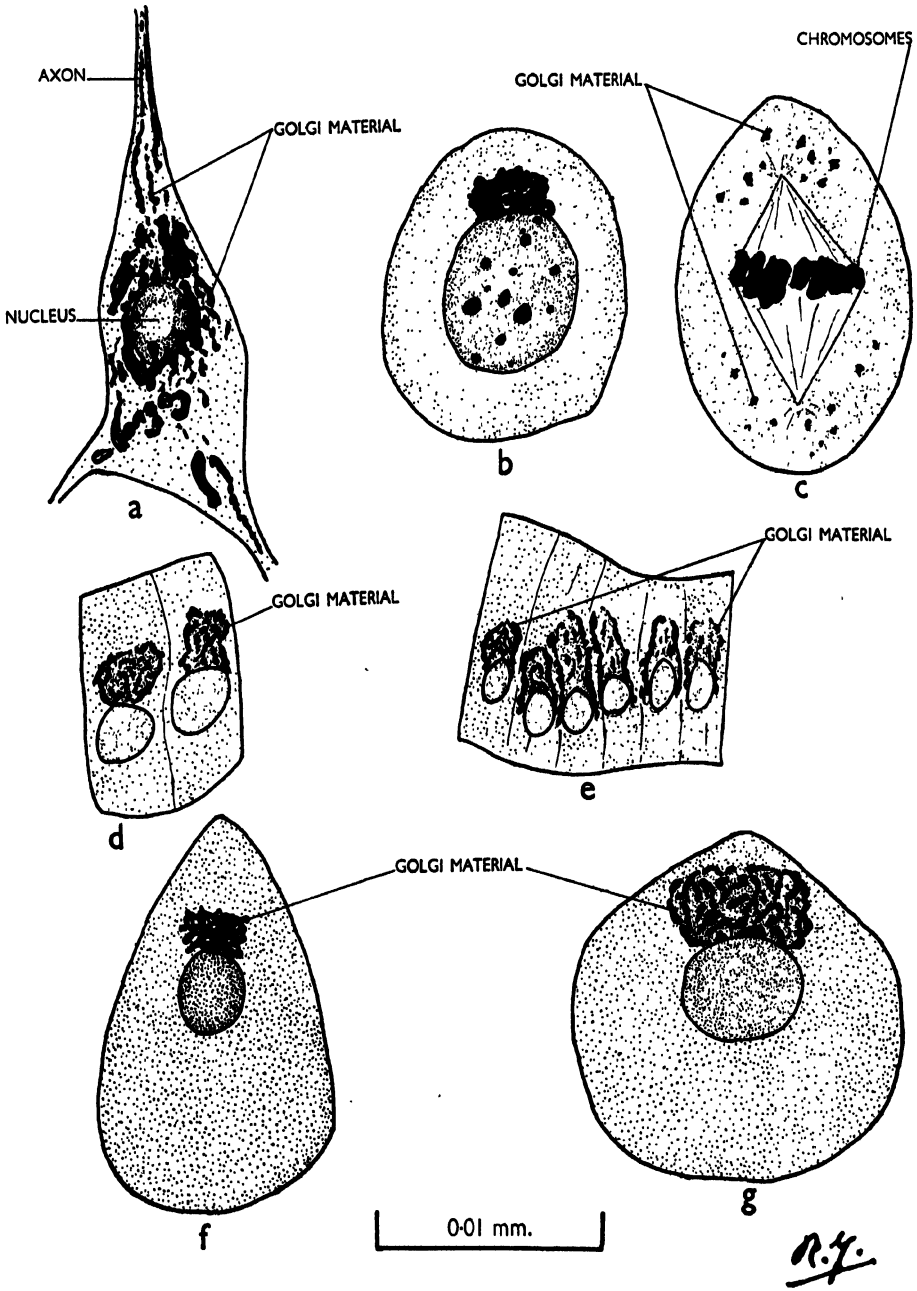

FIG. 61.—a, pyramidal cell from cerebral cortex of sheep. b, primary spermatocyte of the pig to show the Golgi material in the localized condition. c, primary spermatocyte of the pig to show the distribution of the Golgi bodies during cell-division. d and e, cells in stages of secretory activity from different parts of the oviduct of the mouse. f, pancreatic cell of the mouse. The cell is not active. g, pancreatic cell of the mouse. Secretory granules are being formed ; the Golgi material is hypertrophied. Original drawings.

to fat extraction after freezing and drying, and believes that the Golgi substance contains diffuse protein and faint traces of lipoidal material.

A few workers have denied the existence of Golgi material and believe that the structures present in osmic and silver preparations are artifacts, or else that fat blackened with osmium has been described as Golgi material. Its occurrence, however, in practically every type of vertebrate tissue, its identification in the cells of many invertebrate animals, and its presence in ultra-centrifuged cells leaves little doubt that it is a definite cell component. It is claimed that it is visible in living cells suitably examined, and that it is present in tissue culture cells. Bodies which are probably Golgi elements have recently been revealed by *electron microscopy* (Porter, Claude and Fullam, 1945).

The Golgi material is displaced in ultra-centrifuged cells, and observations indicate that it varies in consistency in different types of tissue. Beams and King (1938) state that the fat globules in the egg of the guinea-pig are thrown to the *centripetal pole* and that the Golgi material usually forms a layer in the *centripetal half*, or in the middle of the cell, but in about 25 per cent of the ova examined it is present in the centrifugal layer of mitochondria. In the oocytes of *Lumbricus* (Normington, 1937) and of the pigeon (Singh, 1938) fat globules are present at the *centripetal pole* and the Golgi substance forms a layer between the fat and the mitochondria. In the ultra-centrifuged adrenal gland of the rat, according to Dornfeld (1936), the Golgi material of the medullary cells is present as a network *centripetal* to the nucleus, and in the cortical cells it is often broken up into granules. In ultra-centrifuged spinal ganglion cells (Brown, 1936) the Golgi material is displaced, but is relatively rigid and retains its form. In the young oocyte of the mouse (Gresson, 1940) the localized Golgi material is displaced as separate elements.

There is reason to believe that the Golgi material consists of an outer *argentophilic* and *osmiophilic* part and an inner *argentophobic* and *osmiophobic* region, and that it is made up of protein and lipoids. Hirsch (1939) believes that the Golgi material is not always differentiated into these two regions but may be present in a condition which he calls the Golgi "*pre-substance*", and which later assumes a double structure with an argentophilic and osmiophilic *externum* and an argentophobic and osmiophobic *internum*. The "pre-substance" stains with neutral red and with Janus Green B. The separate pieces may become associated together to form a network; the true Golgi substance, however, is never in the form of a network. The view that the Golgi material is identical with a system of clear canals, first described by Holmgren, is not now widely held, and Brown (1936) has shown that the canalicular system of vertebrate neurones is unaffected by the ultra-centrifuge, while the Golgi material is displaced towards the centripetal end of the cell.

Some workers have claimed that the vacuoles or the plastids of plant

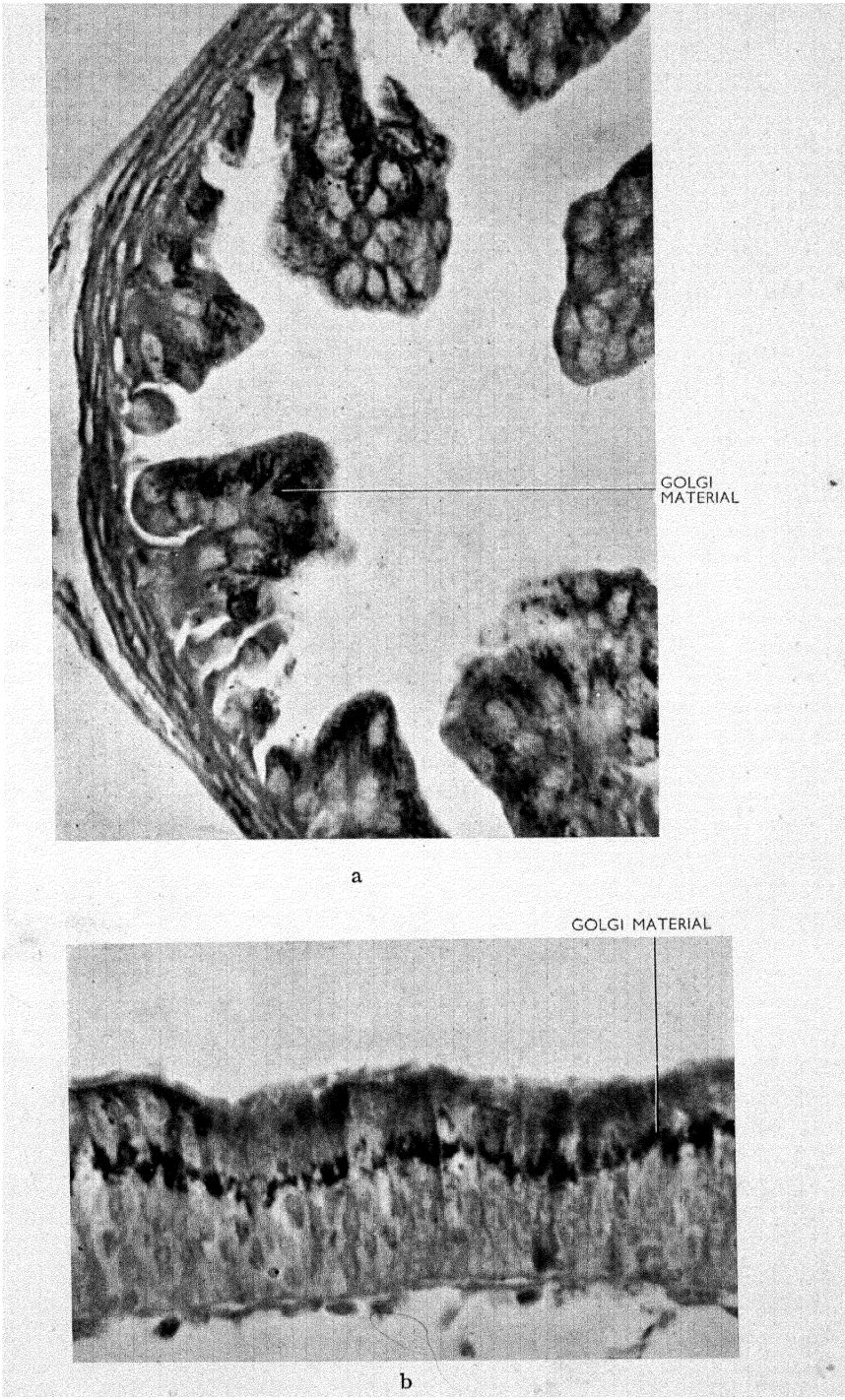

FIG. 62.--Photomicrographs. a, part of the oviduct of the mouse showing the Golgi material. b, part of the duodenum of a young fowl. The Golgi material is situated between the nucleus and the lumen. During secretory activity the Golgi material hypertrophies (Personal communication from Dr. K. Chodnik). × 575.

cells (p. 29) are homologous with the Golgi material of animal cells, but such views are not generally accepted. Certain cytologists believe that the osmiophilic platelets of plants (p. 29) represent Golgi substance, and it is noteworthy that, when cells of the bean root-tip are ultra-centrifuged, the platelets, like the Golgi material of animal cells, form a layer in the centripetal part of the cell, while the plastids are thrown to the centrifugal pole (Beams and King, 1935).

According to the vacuome theory of Parat and Painlevé (1924) the Golgi material is an artifact produced by the precipitation of osmium or of silver inside, or at the surface of neutral red vacuoles. Later, Parat (1928) stated that the vacuoles were surrounded by modified mitochondria which he called the " chromoriome actif ". It is now known that in the living cell various granules and vacuoles stain with neutral red, and that the dye, when introduced into living tissue, often appears to be segregated into vacuoles. Consequently, although vacuoles have frequently been observed in topographical relationship to the Golgi material, considerable doubt has been expressed as to the accuracy of Parat's interpretations. Recently, however, Baker (1944) has given an account of the structure and arrangement of the Golgi material which agrees in many respects with the conceptions of Parat and his co-workers. Baker states that Golgi material is destroyed or imperfectly preserved by all fixatives containing protein precipitants, and claims that a true picture of its structure is given by fixation with formal in calcium carbonate solution and subsequent staining with sudan black. He examined the primary spermatocytes and early spermatids of *Helix aspersa*, the absorptive cells of the intestinal epithelium of *Triturus vulgaris*, and the cells of the anterior mesenteric ganglion of the rabbit. He calls the localized Golgi material the *Golgi element*, and believes that the fully developed Golgi element consists of vacuoles with fluid content which stains with neutral red, a *dense lipoid-containing* substance, usually in close association with the vacuoles, and a *diffuse lipoid-containing* substance which fills the region occupied by the Golgi element (fig. 63). The dense lipoid-containing substance assumes different forms in different kinds of cells, and sometimes in the same type of cell. It may surround, or partly surround, separate vacuoles, or two or more vacuoles; it may appear as irregular strands extending from vacuole to vacuole and spreading out over part of their surface; it may form rods which are on the surface of the vacuoles, or which may not be closely associated with the vacuoles, and in some cases it forms a ring round a vacuole.

Baker states that some of the forms assumed by the Golgi element, and the Golgi net of nerve cells, are probably artifacts, and that the network of other cells is often an artifact. He believes that the vacuoles are destroyed in material fixed by the usual methods and that the lipoid-containing substance is left as a network. The diffuse lipoid-containing

substance is not so strongly sudanophil and osmiophil as the dense substance, and is sometimes absent from the cells of invertebrates and

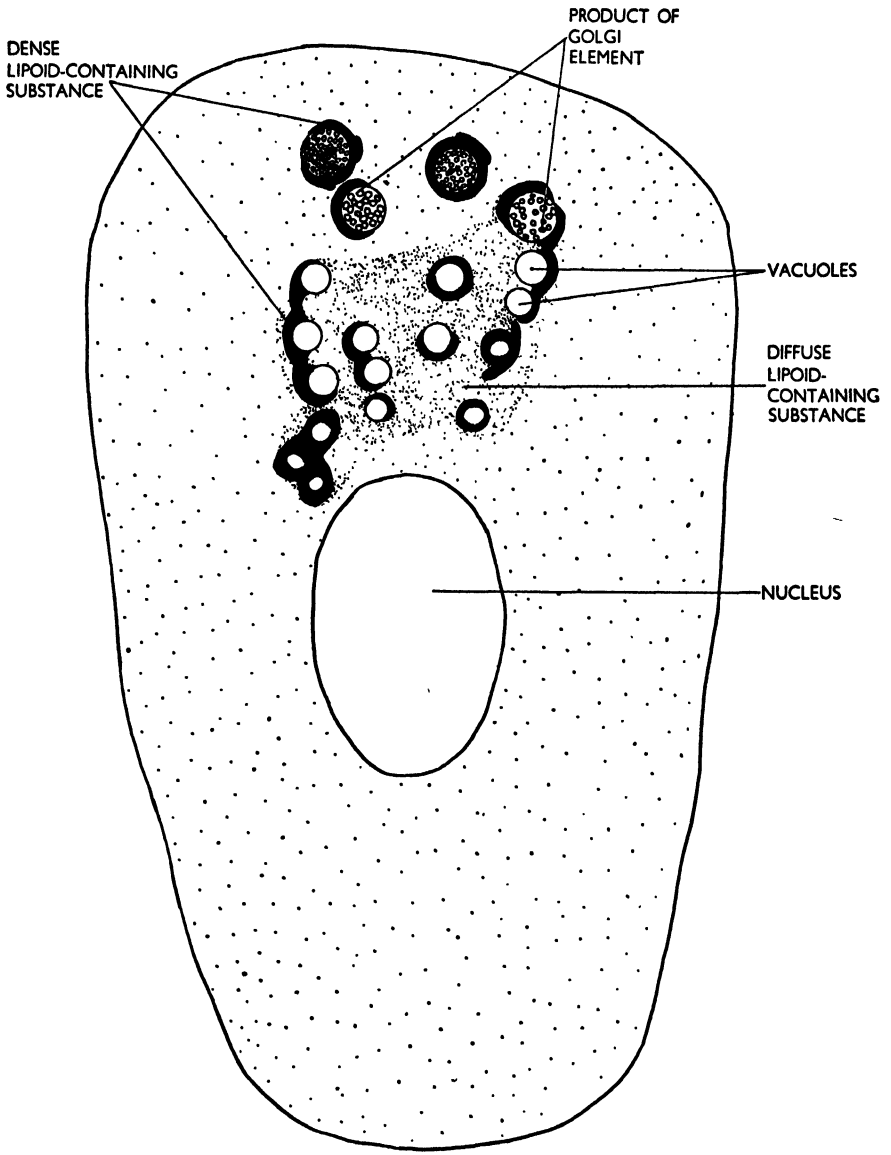

FIG. 63.—Diagram illustrating the structure of the Golgi element. After Baker, redrawn and modified.

often from those of vertebrates. The diffuse and the dense substance together form the Golgi externum. The synthetic product of the Golgi element appears within the vacuoles and, when formed, moves away from the Golgi zone. In cells which are not functioning actively the vacuoles

are absent, or are so small that they cannot be distinguished; this condition represents the Golgi " pre-substance ".

Vitamin C is often present in the part of the cell occupied by the Golgi material. For example, Barnett and Bourne (Bourne, 1942) claim that vitamin C appears to be localized in the region of the Golgi substance of the tissues of the chick embryo, and, according to Hirsch (1939), if strips of the intestine of starved *Ascaris* are incubated in 0·1 per cent solution of vitamin C, the vitamin is absorbed and concentrated in the internum of the Golgi material. When fat is forming in fibroblasts the Golgi material breaks up into granules which become scattered through the cell; the granules often become attached to the fat globules and vitamin C is said to have a distribution similar to that of the Golgi granules. Work on kidney tubule cells indicates that vitamin C is segregated by the Golgi material, and later migrates across the cell either in the Golgi substance or in the form of granules (Bourne, 1942). These observations, and other work, indicate that vitamin C is associated with the Golgi material of different tissues, and that it is often concentrated in the Golgi substance of cells in which synthesis of various materials is proceeding.

That the Golgi material contains lipoidal substance appears to be established, and it is probable that it also contains protein. There is, however, no general agreement as to its morphology. In the opinion of the writer, there is strong evidence that this important cell component is to be regarded as consisting of material which assumes different forms; that it seldom, if ever, exists as a true network, and that it has a double structure with an argentophilic and osmiophilic externum and an argentophobic and osmiophobic internum. Baker believes that the dense lipoid-containing substance assumes different forms and that neutral red vacuoles are present. It must be understood that different substances in the living cell are stained by neutral red, and hence that all neutral red vacuoles and granules are not necessarily associated with the Golgi material. Kirkman and Severinghaus (1938), Bourne (1942) and Hibbard (1945) have reviewed the literature dealing with the structure and function of the Golgi material, and these works should be consulted, together with Baker's contribution (1944).

THE MITOCHONDRIA

Mitochondria are present in all types of animal tissue, and may take the form of *granules*, *filaments*, *rods* or *spheres* (fig. 64). The shape of the mitochondria often appears to be characteristic for certain types of cells, but in gland cells, and in other tissues also, they undergo changes of form which are correlated with the activity of the cell. The mitochondria of any one cell are of approximately the same diameter; they grow in length and are said to divide by transverse fission. Rod-shaped mitochondria often break up to form granules, and the granules may come

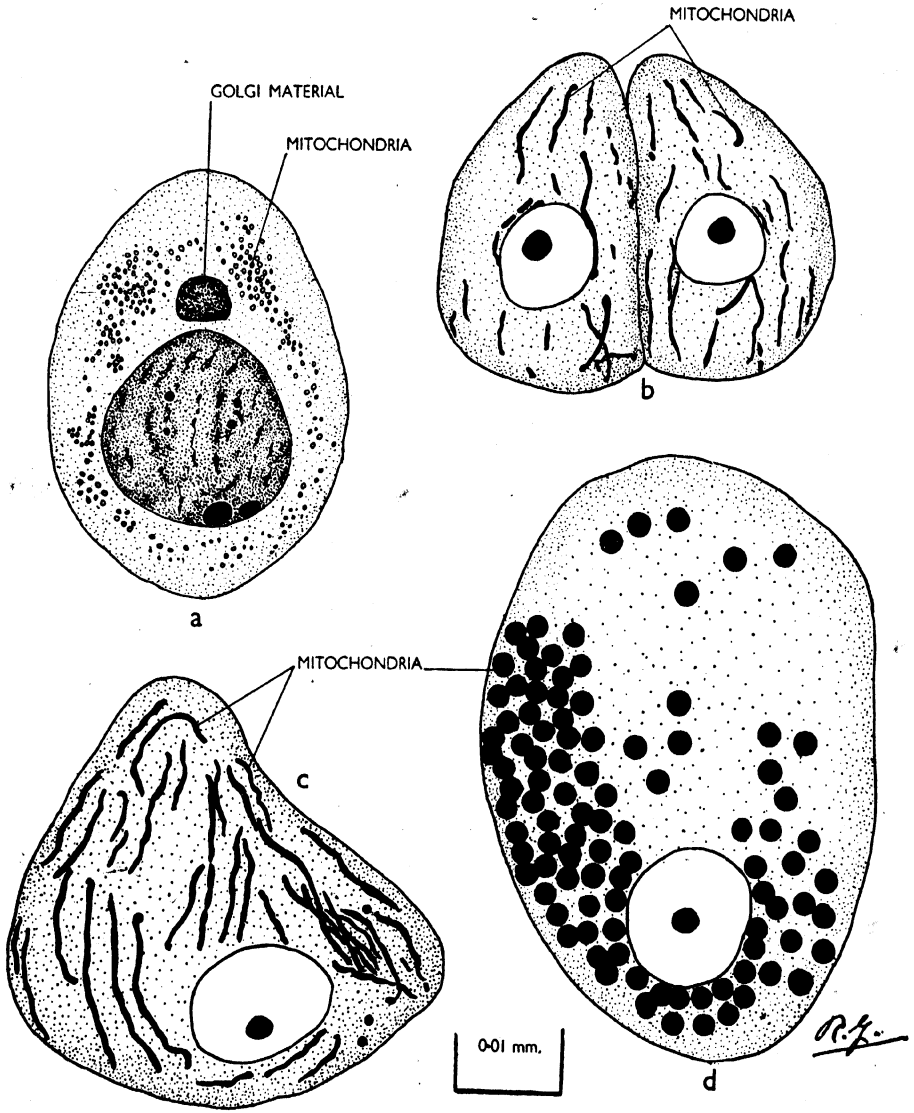

FIG. 64.—Mitochondria. a, primary spermatocyte of the rat. b, two cells from the kidney of a mammal. c and d, cells from a section of the liver of a turtle. In c the mitochondria are in the form of filaments, and in d they are granular ; the change is probably correlated with phases of activity of the cell. Original drawings.

together again to form filaments and rods. In addition to changes of form, the mitochondria undergo active movements; rods and filaments, when observed in living cells, often show a wriggling movement, and mitochondria frequently migrate from one part of a cell to another. At a certain stage in the history of the germ-cells they may closely surround the localized Golgi material and archoplasm, and at a later stage scatter through the cytoplasm. In ultra-centrifuged material the mitochondria are thrown towards the centrifugal end of the cell.

Mitochondria have been observed in the cells of many plants. They may be in the form of rods, filaments or granules, and may be arranged around the nucleus or distributed through the cell. In early meristematic cells the mitochondria are usually scattered through the cytoplasm; in older cells they are concentrated in the vicinity of the nucleus, and finally are distributed through the cell (Cowdry, 1924). In the ultra-centrifuged root-tip of the bean they form a centrifugal layer (Beams and King, 1935).

Tests have shown that the mitochondria contain both lipoid and protein. It is thought that they possess a *lipoid cortex* surrounding a *core* of *protein*, and it is claimed that they contain *vitamins A* and *C*, *proteolytic enzymes*, and other substances. Bourne (1942) has reviewed the more recent work on the structure of the mitochondria, and suggested that they possess a *surface film* which is a *mosaic* of *protein* and *fatty* substances and contains some *lecithin* and *cephalin*, a *cortex* containing *protein*, *fatty material*, *vitamin A*, *cholesterol*, *lecithin* and *cephalin*, and a *medulla* possessing *albumens*, *vitamin C*, *glutathione* and *proteolytic enzymes*.

The mitochondria are destroyed or imperfectly preserved in fixatives containing fat solvents, but Bensley and Gersh (1933) have shown that, if tissue is treated by the *freezing-drying* method, the mitochondria are not affected by subsequent treatment with these substances.

Recent work with the *electron microscope* has shown that the mitochondria in tissue culture cells of chick embryos are favourable objects for study (Porter, Claude and Fullam, 1945). In *micrographs* of osmium fixed and unstained preparations the mitochondria are clearly shown and new structural details are revealed. Examination of the filaments shows that dense masses of material are present in certain regions, and at higher magnifications very minute bodies are seen to be distributed throughout the mitochondria. It is likely that further use of the electron microscope will add greatly to our knowledge of the mitochondria and of other cell components.

Pollister (1941) suggested that the long protein molecules of the hyaloplasm are arranged in rows parallel to the lines of protoplasmic flow, and that the mitochondria are excluded from these channels. This explains the orientation of mitochondria outside the astral rays of leucocytes, and parallel to assumed channels of diffusion situated between the basal and lumen ends of epithelial cells.

CHAPTER XVI

THE FUNCTIONS AND BEHAVIOUR OF THE GOLGI MATERIAL AND MITOCHONDRIA

THERE seems to be fairly general agreement that the Golgi material of gland cells is concerned with the formation of specific secretions, but the part which it plays in the process has been interpreted in different ways. Some cytologists believe that secretion granules arise in association with the mitochondria, and certain work suggests that both Golgi substance and mitochondria are concerned with the elaboration of secretions. Mitochondria and Golgi material are present in cells other than those of glands and take part in various cell activities. In this chapter some of the more recent work is reviewed and modern conceptions regarding the functions of these important cell components is summarized.

THE GOLGI MATERIAL

It has long been known that in fixed and stained preparations of the pancreas the secretion granules are first visible in close association with the Golgi material. In sections of these glands prepared by osmic and silver methods the Golgi substance has the appearance of a network, or of masses of tangled filaments, situated at the pole of the nucleus adjacent to the lumen. The onset of secretory activity is marked by hypertrophy of the Golgi material; the whole mass becomes less compact and spreads out into the surrounding cytoplasm (fig. 65). Small secretion granules are visible between the filaments, and portions of the Golgi substance become free of the main mass and move away from the nucleus. The migration of part of the Golgi material is accompanied by movement of the granules of secretion and their concentration in the cytoplasm close to the lumen. Little of the Golgi material reaches the periphery of the cell and most of it remains in the cytoplasm between the nucleus and the granules. The secretion bodies are finally discharged into the lumen, the cell returns to the resting condition, and the Golgi material again takes up its original position in relation to the nucleus. Not only are the granules first visible in close association with the Golgi substance, but the latter undergoes a change of form and distribution during the phases of secretory activity; clearly the Golgi substance plays some part in the process.

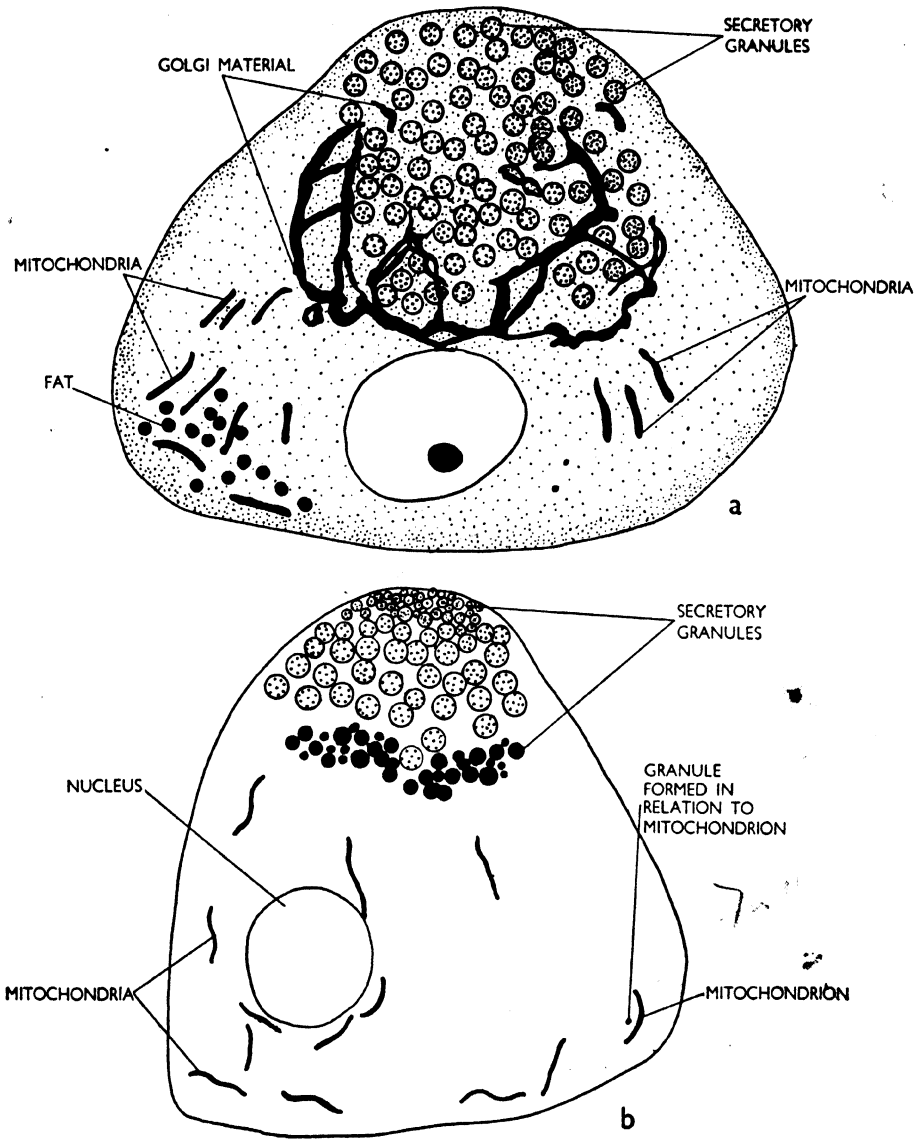

FIG. 65.—a, pancreatic cell of the mouse showing mitochondria, Golgi material. and secretory granules. b, pancreatic cell of the mouse to illustrate the origin and history of the secretory granules. After Duthie, redrawn and modified. a, incorporates features shown in two of Duthie's drawings.

Observations on different types of gland cells have shown that in many cases the granules, or droplets, are first visible in the Golgi zone, but certain cytologists believe that the mitochondria take part in the formation of cell products. The work of Duthie (1933) and of Hirsch (1932) has thrown further light on the relationship of the mitochondria and Golgi material to the origin and growth of secretion granules. Duthie made an incision in the abdomen of an anaesthetized mouse; part of the pancreas was then withdrawn, placed in saline solution on a glass slide, and examined under the high power of the microscope. The blood supply of the pancreas was preserved and it was possible to keep the cells alive for a considerable time. According to Duthie the Golgi substance of fixed preparations of the pancreas of the mouse is in the form of a network situated at the lumen side of the nucleus and surrounding the secretion granules. Filamentous mitochondria are present, a few fat globules are scattered through the cytoplasm, and small granules, similar to secretion granules, are situated towards the basal part of the cell (fig. 65, a). As the result of his observations on living cells Duthie concluded that the granules of secretion originate in the following manner. Small granules, which stain with neutral red, arise in the basal part of the cell in association with the mitochondria (fig. 65, b). The granules remain in contact with the mitochondria for 10-17 minutes and subsequently undergo irregular movements which last for some time. They then migrate towards the Golgi zone where they increase in size; their movement to the Golgi zone takes from 15 minutes to 3 hours. Later, they move towards the lumen, decrease in size, cease to stain with neutral red, and are converted into mature secretion granules. These observations indicate that the granules of secretion originate under the influence of the mitochondria, migrate to the Golgi zone where, under the influence of the Golgi material, they undergo further changes, and finally, as they pass towards the lumen, are converted into mature granules.

Formed products have been identified in association with the Golgi material of many different types of cells of vertebrate animals. In invertebrates evidence of the participation of the Golgi substance in the elaboration of secretion is less complete. For example, in the cells of the mid-gut of *Periplaneta* (Gresson, 1934) granules of secretion are visible close to the Golgi bodies, while in other cases no such association has been observed (Gresson, 1936). Changes in the form and disposition of the Golgi material correlated with the phases of secretory activity have not been observed in the cells of some animals (Hibbard, 1945).

There is considerable speculation as to the precise part which the Golgi material plays in the elaboration of secretion. Kirkman and Severinghaus (1938) believe that the Golgi substance acts as a *condensation membrane* for the concentration of materials which arise elsewhere in the cytoplasm. Many cytologists think that it is concerned with the *synthesis* of various cell products, and there appears to be considerable

evidence in favour of this view. Hirsch (1939) believes that the secretion granules arise in the internum of the Golgi material, and that prior to their origin *vitamin C* is absorbed into the internum and takes part in the formation of the granules. Bourne (1942) thinks that, while the vitamin plays some part in the process, it is unlikely that it takes a direct part in the synthesis of the cell products. Tonutti (Bourne, 1942) suggested that vitamin C is stored in the Golgi material and liberated slowly to prevent oxidation of the products in the cytoplasm, but Bourne believes that a more likely explanation is " that unless these various products are being protected by being produced in, or absorbed onto a specially segregated, highly reducing area of the cytoplasm, they would be oxidized as rapidly as they are formed ". It is claimed that substances other than vitamin C are absorbed by the Golgi material.

✓ Subramanian (1934, 1935 and 1937) believes that the function of the Golgi material is the secretion of *intra-cellular enzymes* and that secretory products, such as fat, yolk, mucus, etc., are secondary products resulting from the action of the enzymes. He thinks that during the oogenesis of *Acentrogobius* the Golgi bodies are initially concerned with the formation of fatty yolk and later with the secretion of mucus. Subramanian, therefore, suggests that the Golgi substance may take part in the elaboration of different materials at different periods in the history of the same cell. He also suggests that the acrosomic vesicle of the spermatid contains enzymes secreted by the Golgi material.

In the eggs of some animals, chiefly invertebrates, the Golgi material is described as being in the form of *vesicular bodies* consisting of an osmiophilic rim surrounding a clear area. In certain cases it is thought that fatty yolk is laid down in the clear area and that some of the Golgi bodies are finally transformed into fatty yolk globules (Gresson, 1933; and Nath, 1933). According to Worley (1944) vesicles present in the living eggs of *Mytilus* stain with methylene blue and are osmiophilic in fixed preparations. He identified the vesicles as Golgi bodies and stated that they give rise to fat or to protein yolk, or to both. In the ova of some vertebrates, it is stated, fatty yolk is formed under the influence of the Golgi elements, and mitochondria are transformed into protein yolk (Bhattacharya and Lal, 1929), while certain workers believe that fatty yolk is secreted by the ground cytoplasm (Singh, 1938). In some animals, it is claimed, the Golgi material is concerned with the formation of protein yolk (Harvey, 1931). In different types of degenerating cells the Golgi substance is converted into fat. It is possible, therefore, that the Golgi elements of the eggs of some animals may give rise to fatty yolk, but the conversion of their substance, in healthy cells, into other materials appears to be a rare occurrence. Cramer and Ludford (1925) stated that the Golgi material of intestinal cells is concerned with the synthesis of fat from absorbed fatty acids and glycerol.

To summarize: The Golgi material is an important cell component which undoubtedly plays a part in the formation of a wide variety of cell products; there is, however, no general agreement as to whether it acts as a condensation membrane or is concerned with the synthesis of material inside its substance or externally in the cytoplasm. There is strong evidence that it is concerned with the elaboration of substances which are formed by its synthetic action, or possibly by the production of intracellular enzymes. Its presence in practically every type of animal cell, and its established importance in many of those which have been more closely examined, suggests that it has different functions in various tissues.

THE MITOCHONDRIA

It has been suggested that the mitochondria are centres of cell respiration, and while this suggestion cannot be dismissed, the evidence in its favour appears to be inconclusive. There is reason to believe that they play an important part in the formation of secretions, and that enzyme activity takes place at their surface. It is also probable that the mitochondria are concerned with yolk-formation. Several of the earlier workers believed the mitochondria of embryonic cells to be transformed into myofibrils and neurofibrils, but more recent work does not support this view. It has also been claimed that young plastids (p. 126) resemble mitochondria, and that in embryonic plant tissues mitochondria become enlarged and are transformed into plastids.

Horning studied the mitochondria of some of the Protozoa and believes that in *Amoeba* they are concerned with the production of enzymes which bring about the digestion of the food. He also claimed that in *Opalina* protei[illegible]granules are formed under the influence of the mitochondria (p. 150). It would appear, therefore, that the mitochondria of the Protozoa are concerned with enzyme activity and with the synthesis of certain formed products.

That the mitochondria play a part in the formation of secretion granules in multicellular animals is claimed by certain cytologists. The evidence that they take a direct part in the process is, for the most part, less convincing than in the case of the Golgi material. An interesting contribution, claiming that both Golgi substance and mitochondria are concerned with the elaboration of the secretion of the mammalian pancreas, is discussed in the section dealing with the functions of the Golgi material (p. 138).

The mitochondria are connected with cell functions other than those of secretion and yolk-formation. They form the sheath of the axial filament of the sperm middle-piece, and it is possible that the mitochondria of which it is composed play some part in the physiology of the spermatozoon. It is probable that in non-secreting cells the mitochondria may take

part in the formation of some of the cytoplasmic constituents and in the general metabolism of the cell.

THE MITOCHONDRIA AND GOLGI MATERIAL DURING CELL DIVISION

The manner of the distribution of the mitochondria during cell division varies in different organisms and in different cells of the same organism. Granular mitochondria may be more or less evenly distributed throughout the cytoplasm, as in the spermatocyte divisions of the rat (fig. 66, a) or they

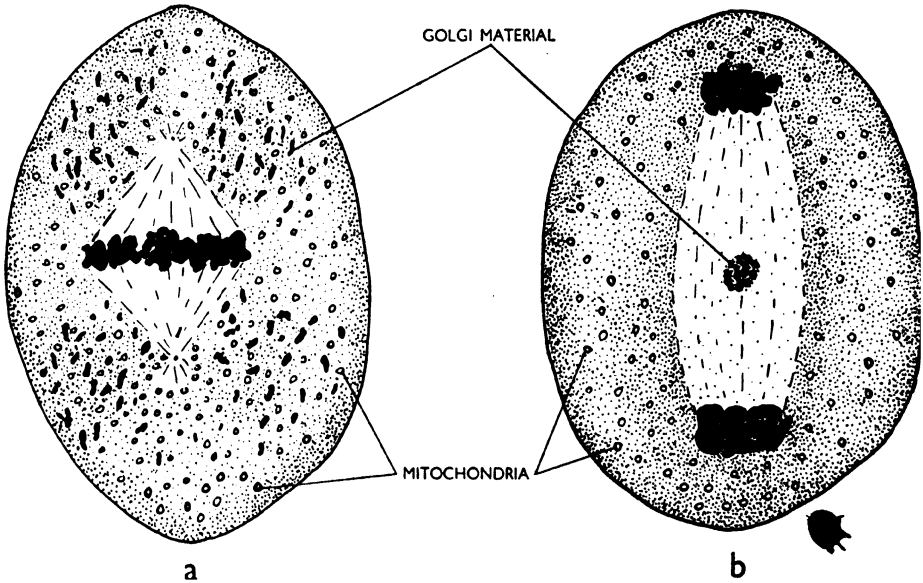

FIG. 66.—a, primary spermatocyte of rat. After Gresson and Zlotnik, redrawn and modified. b, primary spermatocyte of mouse. After Gresson, redrawn and modified.

may be concentrated around the spindle (fig. 40, c). In the spermatocytes of some insects granular mitochondria run together to form threads which lie around the equatorial region, and each thread is subsequently divided into two parts by the division of the cytoplasm. In the primary spermatocytes of *Centrurus* the granular mitochondria aggregate to form a ring-shaped body which, by the division of the cell, is separated into two half-rings. Each of the half-rings divides to form two rod-shaped bodies which are separated into two shorter rods by the division of the second spermatocyte. By this means each of the spermatids receives two rod-shaped mitochondria. The examples cited above show that in some animals the mitochondria remain scattered through the cytoplasm, while in others they take up a definite position in relation to the spindle. In either case

the division of the cytoplasm results in their distribution in approximately equal numbers to the daughter cells.

Approximately half the Golgi elements of the spermatocytes of the guinea-pig are arranged around each pole of the spindle. During the anaphase of the spermatocytes of the opossum (Duesberg, 1920) the Golgi material breaks up into granules which form one or more clumps between the chromosomes. In the mouse (Gresson, 1942) a clump of Golgi elements is situated in the equatorial region during the metaphase, anaphase and telophase, and is apparently separated into two parts by the division of the cell (fig. 66, b). During the first cleavage division of the egg of the mouse the Golgi elements are scattered through the cytoplasm (Gresson, 1941). Gresson and Zlotnik (1945) followed the behaviour of the Golgi material during the spermatocyte divisions of seven mammals and found that, although there were slight differences between the animals studied, in all cases the daughter cells received an approximately equal amount of Golgi substance.

The arrangement of the Golgi elements during the stages of cell division varies in different animals, but in all cases they are transmitted in approximately equal numbers to the resulting cells. The behaviour of the Golgi material during mitosis, and its presence in the cytoplasm of the fertilized egg and distribution to the early blastomeres, indicates that it is a permanent cell component.

CHAPTER XVII

AN INTRODUCTION TO THE CYTOLOGY OF THE PROTOZOA

THE members of the phylum Protozoa vary considerably in size, and in many cases parts of the body exhibit a high degree of structural and functional differentiation. In many of the more complex types a certain area is concerned with the intake of food, and regions of the cytoplasm are specialized to carry out definite functions in connection with the life of the organism. In certain types, such as *Amoeba*, the body consists of a clear outer part and a more granular inner region. The substances of the two areas are unstable and movement is brought about by a reversible change of the inner *sol* into an outer *gel*. In some of the Protozoa two or more nuclei exist, and in such forms as *Opalina* certain phases of the life-history are *multinucleate*. As a consequence of the complexity of structure which often exists, and the occurrence of multinucleate forms, differences of opinion have arisen as to whether the Protozoa are to be regarded as *unicellular* or as *non-cellular* organisms. It is not proposed to enter into the controversy here, and in this chapter the body of a protozoon is regarded as corresponding in general to the single cell of a multicellular animal or plant. It is to be noted, however, that frequently the protozoan cell is highly specialized and internally differentiated and that certain of the Protozoa possess chlorophyll and other plant-like characters.

A vast amount of research has been carried out on the structure of the Protozoa and on problems connected with their life-history and reproduction; a considerable amount of work, however, remains to be done on their minute structure. In recent years Gatenby and his students, in attempting to homologize structures present in certain Protozoa with the Golgi material of the metazoan cell, have made important contributions to our knowledge. It is not possible within the confines of this chapter to give a detailed description of the cytology of the Protozoa, nor to deal with the problems of mitosis and meiosis. Such accounts will be found in works on protozoology (Wenyon, 1926; Calkins, 1933; and Calkins and Summers, 1941) and in the other references listed. The following accounts are intended to serve as introductions only.

MITOSIS

The structure of the fixed and stained protozoan nucleus varies. It usually contains *nucleoli* and often shows a *nuclear network*. Frequently the stained material is concentrated in the central region of the nucleus to form a body which is known as the *endosome*, or *karyosome*, and which may contain chromatin as well as other material. In many of the Infusoria nuclei of two kinds are present; the *macronucleus* is large, is said to divide amitotically, and appears to be concerned with the physiological activities of the cell; the *micronucleus* is small, divides mitotically and is concerned with reproduction.

The method of *nuclear division* varies (fig. 67). In many forms division is simple in character and was formerly described as amitotic, but is now regarded as a simple type of mitosis. Amitosis appears to take place in the macronuclei of the Infusoria only. The simpler kind of mitosis is connected by a series of increasing complexity with a method of division in which the chromosomes behave like those of the metazoa. The following is a convenient method of classifying the main types of mitosis, but other classifications exist. In *cryptomitosis* distinct chromosomes are not present; the chromatin becomes concentrated into a mass at the equator of the spindle, and divides into two smaller masses which move to opposite poles (fig. 67, c–e). In *paramitosis* the chromosomes do not shorten in the metaphase, and the daughter halves remain in contact end to end until a late stage of mitosis so that they appear to divide transversely (fig. 67, a and b). The behaviour of the chromosomes is often like that of the higher animals and plants, and in these cases nuclear division is spoken of as *eumitosis*. In many Protozoa the chromosomes are favourable objects for study and have been shown to possess essentially the same structure as the chromosomes of the multicellular organisms.

The *division centres* and *spindle* present problems of special interest. The division centres may be centrosomes with or without a *centriole*, or centrioles alone may be present, and in some cases plate-like structures only are present at each pole of the nucleus. In many cases the division centre is cytoplasmic in origin, and in the Flagellata is associated, or identical, with the *basal granule* of the flagellum. The basal granule, or *blepharoplast*, is often connected by fibrillae with a larger body known as the *parabasal body*; these structures probably belong to the *neuromotor apparatus*. In some flagellates the blepharoplast acts as a division centre, in others a separate centriole is present; in certain cases the basal granule is closely associated with the centriole and, during mitosis, divides into two parts which move with the centrioles to the spindle poles. It has been suggested that the centriole sometimes acts both as a division centre and basal granule, and that in other cases it has separated into a division

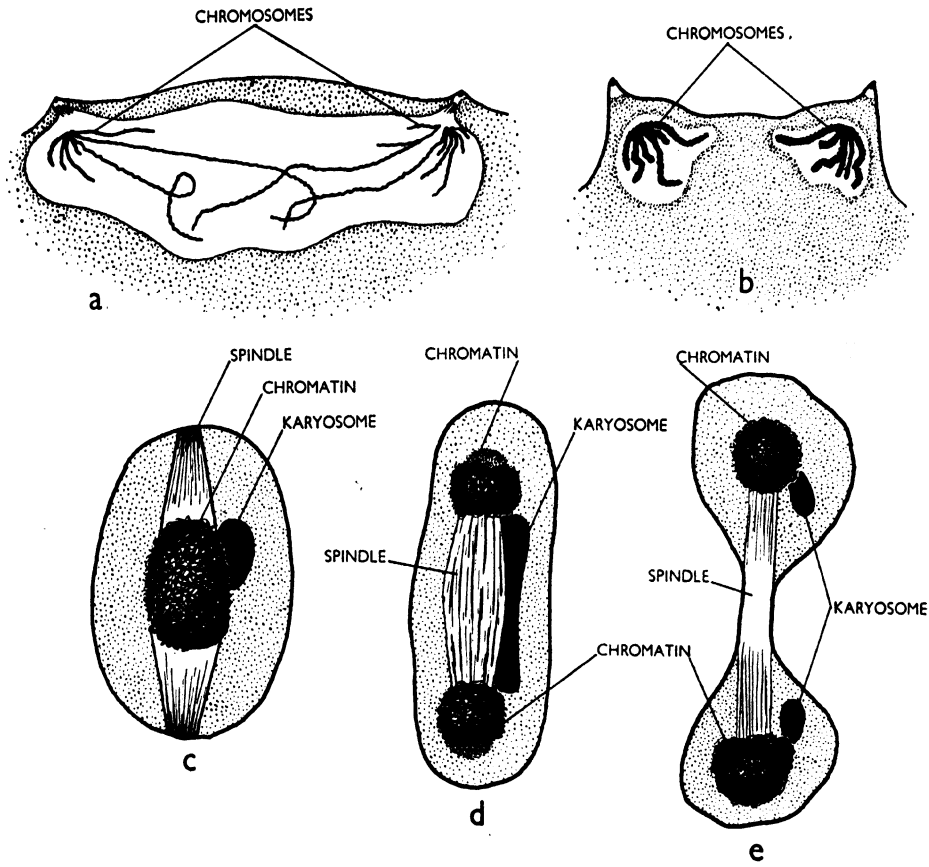

FIG. 67.—a and b, paramitosis in *Aggregata eberthi*. a, early telophase. b, late telophase. After Belar, redrawn and modified. c-e, cryptomitosis in *Haplosporidium limnodrile*. c, metaphase. d, anaphase. e, telophase. After Granata, redrawn and modified.

centre and a blepharoplast. In this connection it should be remembered that, in the sperm of multicellular animals, a centriole acts as a basal granule for the axial filament of the tail (p. 56). In some of the Protozoa the division centre is *intra-nuclear* in origin and often appears to be associated with the endosome. The structure known as the endosome appears, however, to vary in composition; in certain forms it gives origin to the division centres, in some it is said to contain chromatin during the non-dividing phase of the nucleus, while in others it does not appear to contain chromatin at any stage of its history.

The spindle varies in origin and may be derived either from nuclear or extra-nuclear substance. Very often the nuclear membrane remains intact during all the stages of mitosis.

REPRODUCTION AND MEIOSIS

Reproduction in the Protozoa is by cell division; frequently a process of *syngamy* takes place at some stage in the life-history and precedes a new cycle of growth and cell division. In syngamy union is brought about between two cells which are alike, or else are unequal in size and have undergone physiological differentiation. When the gametes are alike they are as large as, or larger than, the ordinary individuals of the species. When they are unequal in size one of the gametes is small and may be regarded as male, while the other is relatively large and passive and may be looked upon as female. In some cases, particularly in the Infusoria, nuclear fusion is not followed by fusion of the cytoplasm. In *Paramoecium*, and other infusorians, two individuals come into temporary association, and after a series of nuclear divisions one of the products (male) of the *micronucleus* of each conjugant migrates into the other individual and fuses with another of the products (female) of the *micronucleus*. The conjugants then separate and the *zygote nucleus* of each divides; new individuals are formed, each provided with a macronucleus and one or more micronuclei, as is characteristic of the species. In many of the Protozoa fusion only appears to take place between gametes which have originated from different individuals, but in *Actinophrys*, for example, *autogamy* takes place. An individual divides into two parts, the nucleus of each part undergoes maturation and the two cells reunite. One of the gametes plays an active part in the process, putting out pseudopodia towards the cell with which it fuses.

In most cases the nucleus of the *gametocyte* undergoes two maturation divisions with reduction of chromosome number (*gametic meiosis*). In certain of the Sporozoa the chromosome number is reduced from the diploid to the haploid during the first division of the zygote nucleus (*zygotic meiosis*). The zygote nucleus, therefore, contains the diploid number of chromosomes, and the haploid number is present throughout

all the other phases of the life-history. Fertilization (Turner, 1941), sexuality (Sonneborn, 1941) and inheritance (Jennings, 1941) in the Protozoa is discussed in recent reviews.

OSMIOPHILIC STRUCTURES AND GOLGI MATERIAL

Modern work suggests that certain of the osmiophilic bodies observed in the Protozoa may be homologous with the metazoan Golgi substance.

Golgi bodies are present in the Sporozoa, and in ultra-centrifuged gregarines they are stratified in the centripetal end of the cell immediately below the fat, where they form an upper layer of fine granules and a lower layer of larger bodies (fig. 68). Brown (1930) claims that in *Amoeba* two kinds of Golgi bodies are present, granules and spheres with osmiophilic rims. Mast and Doyle (1935 (a) and (b)) believe that refractive spheres which stain with neutral red and possess an outer osmiophilic part represent the Golgi bodies. Singh (1938) studied ultra-centrifuged amoebae and states that the granules described by Brown are mitochondria, and that the bodies seen by Mast and Doyle are nutrient spheres. According to Singh, Golgi material is not present in *Amoeba proteus*. Much work has been done on the osmiophilic material of the Ciliata where in many cases the contractile vacuole possesses a cortex which blackens with osmium. In some ciliates granular Golgi bodies occur scattered through the cytoplasm, and Smyth (1944) points out that the osmiophilic cortex of the contractile vacuole of some of these organisms is granular, and concludes that the two types of Golgi material (osmiophilic cortex and granules) are probably closely related. Osmiophilic material is associated with the contractile vacuole or with the reservoir of many of the Flagellata. This is not the case in other members of the group, and certain workers consider that the parabasal body is the homologue of the Golgi material.

Nassanov (1924 and 1925) considered that the contractile vacuole, together with the osmiophilic substance associated with it, represents the Golgi material of the multicellular animals. Gatenby (1938) believes that the osmiophilic material alone is the homologue of the Golgi material. He suggests that the Golgi material originated in connection with the base of the flagellum of some primitive flagellate; became associated with the contractile vacuole of the higher flagellates, and persists in the metazoan cell where it is concerned with the formation of certain cell products. Smyth (1945) found that in the flagellate, *Astasia harrisii*, an oval mass of osmiophilic material is connected with the reservoir by a canal possessing osmiophilic walls. He suggests that this substance is the homologue of the Golgi material, and that it may have been derived from the primitive type by the enlargement of the parabasal body and the formation of a canal. Further, if the material became associated with a contractile vacuole and the connection with the reservoir lost, the condition

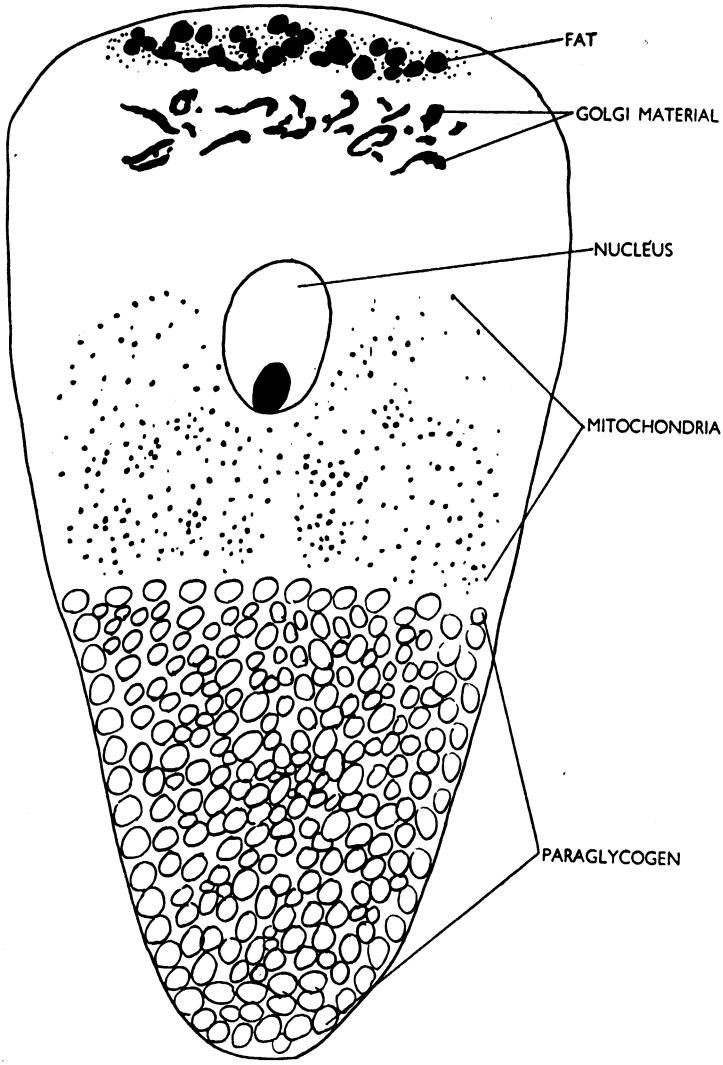

FIG. 68.—Generalized diagram of ultra-centrifuged gregarine. After Daniels, redrawn and modified.

might be comparable with a simplified form of the condition found in *Paramoecium* where several canals with osmiophilic walls lead into the contractile vacuoles. The condition in *Astasia* might, therefore, fit into the line of evolution suggested by Gatenby and help to bridge one of the gaps.

There are difficulties in accepting this theory regarding the evolution of the Golgi material, and much work remains to be done, but Gatenby and his students have focused attention upon an important problem. In support of his conclusions, Gatenby pointed out that Duboscq and Grassé believe that the parabasal body is the homologue of the Golgi material, that the work of several observers shows that the Golgi substance of the flagellated cells of sponges lies in close topographical relationship to the centriole flagellar apparatus, and that, in the non-flagellated somatic cells and reproductive cells of these organisms, the Golgi material closely resembles that of the higher animals.

There have been relatively few observations on the behaviour of the osmiophilic material of the Protozoa during mitosis. A substance which blackens with osmium surrounds the contractile vacuole of *Chilomonas* (Gatenby, 1941). In about 70 per cent of the dividing individuals examined, the osmiophilic substance surrounding the contractile vacuole was seen to separate into two parts, one of which enters each of the daughter cells. In about 3 per cent the osmiophilic material passes whole into one of the new individuals, while the contractile vacuole of the daughter cell which does not receive this substance acquires a new cortex. In *Vorticella* the contractile vacuole and associated osmiophilic material remains in the daughter cell provided with a stalk; in the other individual a new vacuole arises and later acquires a cortex which blackens with osmium and is probably formed from scattered osmiophilic granules. Smyth (1941) found that, in the ciliate *Linotus*, the scattered Golgi bodies divide and half the number present in the dividing cell is passed on to each of the new individuals.

MacLennan (1941) discussed the osmiophilic structures of the Protozoa and remarked that Golgi bodies have been demonstrated in all stages of the life-cycle of certain Sporozoa and their origin from pre-existing Golgi material described; in other members of the group, he stated, the Golgi elements are not self-perpetuating. As the Golgi bodies of the multicellular animals are believed to be self-perpetuating, and as there is uncertainty concerning the homologies of the osmiophilic structures of some of the Protozoa, it appears to the writer to be unsafe, at this stage of our knowledge, to conclude that definite Golgi bodies arise *de novo*.

MITOCHONDRIA

Mitochondria have been identified in many Protozoa, and may consist of granules, rods or spherules, but filaments are not usually found

(MacLennan, 1941). In most cases they are scattered fairly evenly throughout the cytoplasm, but in some of the Protozoa they tend to concentrate around storage granules, contractile vacuoles, and near other membranes. It is claimed that they divide, and in certain ciliates are said to do so synchronously with the nucleus. In ultra-centrifuged material they are separated from the other cell inclusions, and in gregarines (fig. 68), for example, form a layer between the Golgi bodies and paraglycogen granules (Daniels, 1938).

According to Horning (1926) the mitochondria of *Amoeba* become associated with the ingested food particles, and a vacuole is formed which encloses a food particle and the adjacent mitochondria. Other mitochondria now collect on the outside of the vacuole, and as digestion of the food takes place the mitochondria become smaller and finally pass into solution. They are, therefore, apparently concerned with the production of enzymes. In *Opalina*, protein granules appear at the surface of mitochondria, and Horning (1925) believes that the granules are synthesized under the influence of the mitochondria.

It has been claimed by different workers that the mitochondria of the Protozoa have a respiratory, secretory, digestive or excretory function. MacLennan (1941) concludes that the mitochondria do not form a homogeneous group, but that " no one type is found in all Protozoa, and in all cases which have been carefully studied mitochondria are not self-perpetuating but arise *de novo* at some time during the life cycle ". If they are not self-perpetuating they are unlike those of the multicellular animals, and in the opinion of the writer it is possible that in some of the Protozoa other structures have been mistaken for mitochondria.

CHAPTER XVIII

THE CYTOLOGY OF DEGENERATING AND PATHOLOGICAL ANIMAL CELLS

THE behaviour of the nuclear and cytoplasmic components has been studied under a variety of pathological conditions. Already cytological examination of diseased tissues has produced information of value, and as the cell is the basic functional unit of the body, it is likely that further research in this field will yield knowledge of importance to medical and veterinary science. In this chapter no attempt is made to deal with the grosser aspects of histopathology, nor is it possible to deal fully with recent research in the cytological field. Brief reference is made to some of the channels along which work has proceeded and some of the problems which await elucidation are indicated.

CELLULAR DEGENERATION

The progressive changes which occur during cellular degeneration have been followed in the living cells of tissue cultures in which conditions unfavourable for further growth have been allowed to develop. Similar changes have been observed in pathological tissues. In degenerating tissue culture cells, granules, and in many cases vacuoles and fat globules, appear in the cytoplasm; the formation of the granules and vacuoles is probably due to the accumulation of toxic substances. In certain types of cells the clear area surrounding the centrioles increases greatly in size, and it is probable that its enlargement is due to cytoplasm flowing into it. An increase in the size of the cell and of the nucleus often takes place prior to the disintegration of the nucleus. In the earlier stages of degeneration nuclear buds have been observed passing into the cytoplasm; vacuoles appear round the buds and the latter finally disintegrate. Frequently the nucleus breaks up to form a number of smaller bodies. The Golgi material breaks up and there is evidence that it is converted into fat. The mitochondria collect round the nucleus and the area surrounding the centrioles; later, they break up into granules, some of which increase in size and become vesicular. In the more advanced stages of degeneration the mitochondria are present as fine granules, and, in addition, a few short rods are usually situated at the periphery of the cell; they finally disintegrate.

Mitochondria are believed to be extremely sensitive to abnormal conditions, but those of neurones, and of other cells under certain toxic conditions, are said to maintain their normal form. The mitochondria of degenerating cells are thought by some cytologists to be converted into fat, but this is denied by other workers. It appears that both the mitochondria and the Golgi material of ova in atretic follicles of the mouse are transformed into fat, and there seems to be fairly general agreement that the fat globules present in degenerating tissue culture cells are formed from Golgi material. The Golgi remnant of the spermatid of some mammals, it is claimed, is converted into fat, while in others fat droplets are present in the residual cytoplasm before the Golgi remnant breaks up. It is probable, therefore, that degenerating Golgi material is frequently transformed into fat, but in some cases its conversion into fat globules has not been observed.

PATHOLOGICAL TISSUE

Pathological conditions are commonly followed by degenerative cellular changes, but such changes are often preceded by an increased compensatory activity with hypertrophy of certain cell structures. The morphological changes undergone by cells have been studied in a variety of pathological conditions, but there are still wide gaps in our knowledge. Ludford (1942) states that "no detailed cytological investigation has been made by modern techniques of the reactions of the cells of the principal organs in the case of any bacterial or protozoan infection". The nuclei and cytoplasmic components of malignant cells have been subjected to cytological examination, the action of certain viruses upon cells studied, and the behaviour of cell components investigated under some other pathological conditions. Cowdry (1924) discussed some of the older work on the mitochondria and gave a summary of observations on the Golgi material of pathological tissues. More recently, Ludford (1942) reviewed the literature dealing with the pathological aspects of cytology.

The cells of malignant tumours possess the capacity for unlimited and uncontrolled growth, and transplantation and tissue culture experiments show that these cells maintain their character unaltered for long periods. They often undergo a marked dedifferentiation, but in many cases retain their function, or ability to produce a specific secretion. As tumours have been induced experimentally by X-rays and other means, it has been suggested that certain agents induce *mutations in somatic cells* which result in the production of malignancy. It has also been suggested that cytoplasmic factors may be involved (Ludford, 1942; and Haddow, 1944).

The nuclei of malignant cells may possess the normal number of chromosomes, or wide variations in number, size and shape may exist. Abnormal mitoses, such as unequal distribution of the chromosomes and

suppression of cytoplasmic division, are common, and there is considerable variation in the size of cells even within the same tumour. An increase in cell size is accompanied by an increase in the number of the mitochondria and of the amount of Golgi material. In gland cells which retain their secretory activity the mitochondria are numerous and often elongate, and the secretion granules arise in topographical relationship with the Golgi material.

The cytology of the human thyroid gland in cases of *exophthalmic goitre* has been the subject of numerous investigations. There is general agree-

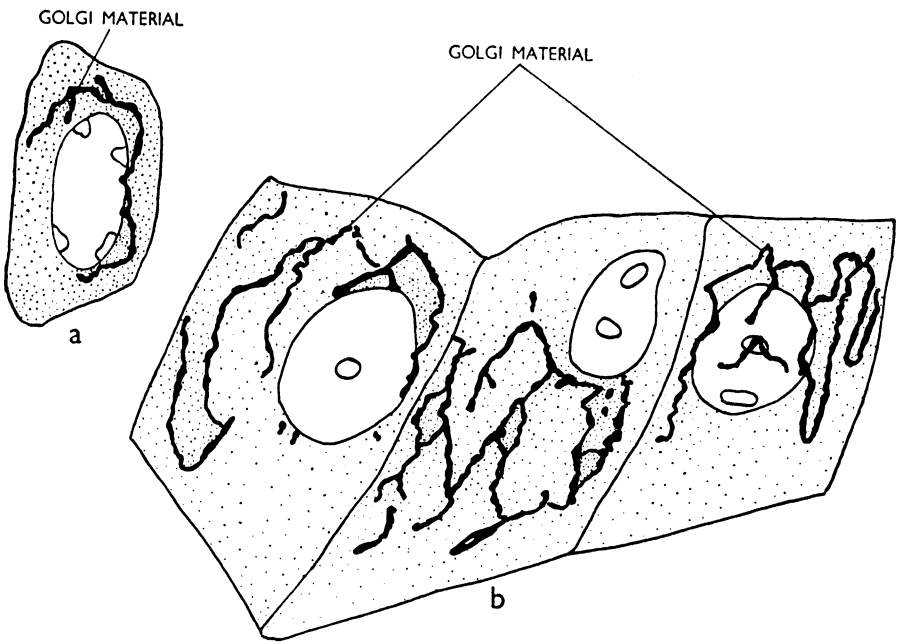

FIG. 69.—Thyroid cell from a case of exophthalmic goitre in a mouse. a, relatively normal cell. b, cells showing hypertrophy of the Golgi material ; in the middle cell the Golgi material has undergone reversal of polarity. From Ludford, after Ludford and Cramer, redrawn and modified.

ment that the mitochondria become long and filamentous, that the Golgi material undergoes hypertrophy (fig. 69), and that these changes indicate increased secretory activity (Ludford, 1942). Welch and Broders (1940) found that in cases of *adenomatous goitre*, not diagnosed as hyperthyroids, the Golgi material of some of the cells was enlarged. Consequently they interpreted such cases as mild hyperthyroidism. Certain workers have found that in hyperthyroidism the Golgi material of many of the cells of the thyroid gland undergoes reversal of polarity, and they believe that this indicates that the secretion of these cells is discharged directly into the blood capillaries and not into the cavities of the alveoli as in the normal

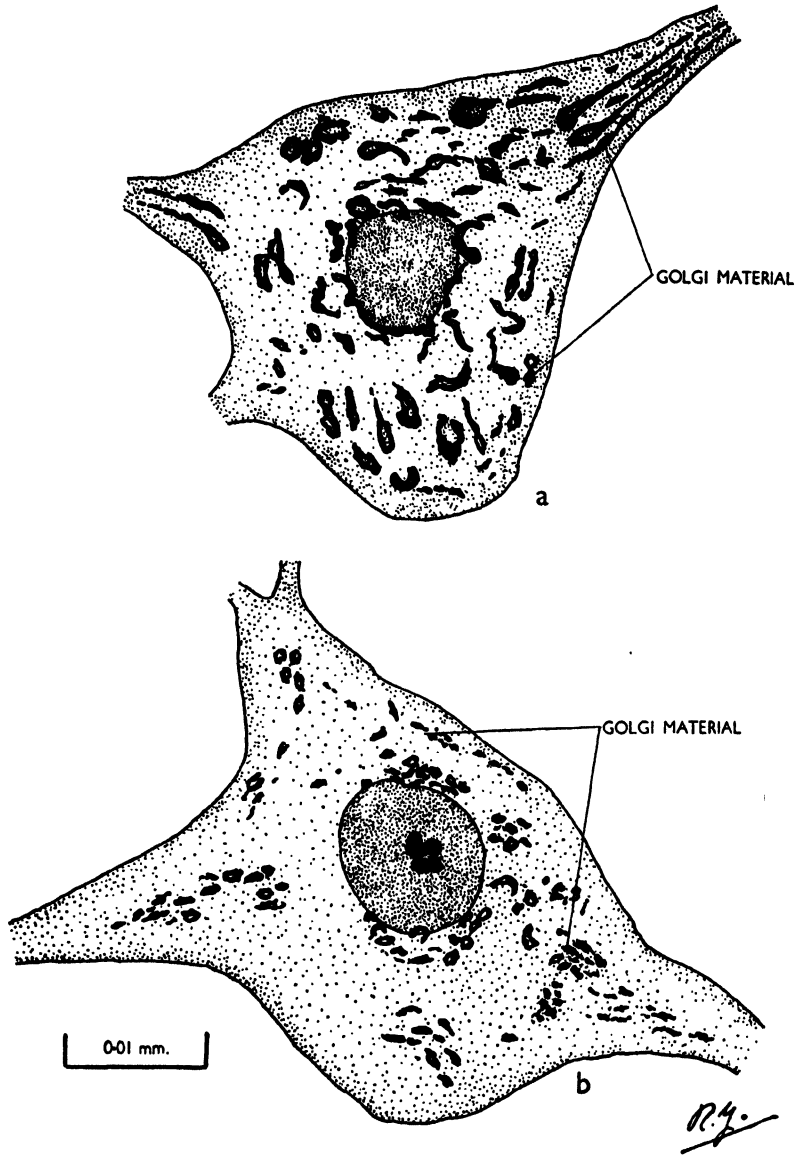

FIG. 70.—Multipolar nerve cells from the spinal cord of sheep. a, normal cell. b, cell from the spinal cord of a sheep infected with the virus of louping-ill. Original drawings.

gland. It has been denied by some workers that the position of the Golgi material in the cell is an indication of the direction in which the secretion is discharged, and that reversal of polarity takes place (Ludford, 1942). It is clear, however, that work on the Golgi material of the thyroid gland, and of other normal and pathological tissues, shows that the study of the morphology and disposition of the Golgi substance may be used to estimate cellular activity. It is of interest that Bailif (1941) found that the behaviour of the Golgi material of *macrophages* in the rat omentum indicates phagocytic response. He claims that, following the injection of certain materials,

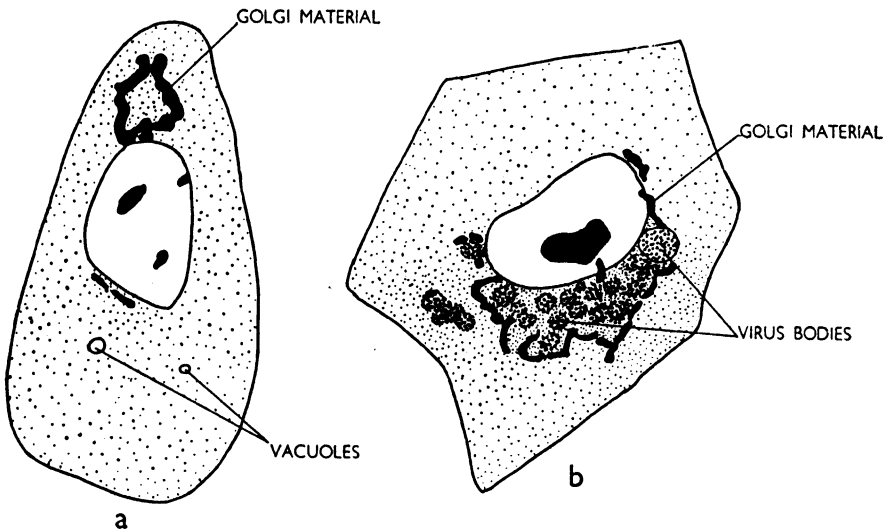

FIG. 71.—Epidermal cells of the chick infected with the virus of fowl-pox. a, cell showing earliest indication of infection. b, cell showing altered polarity of the Golgi material and the formation of virus bodies in relationship with the Golgi material. From Ludford, after Ludford and Findlay, redrawn and modified.

the Golgi substance hypertrophies and breaks up, and that the fragments become associated with ingested granules and apparently aid in bringing about their coalescence into masses which are stored by the cells.

Viruses have been described as ultra-microscopic, filter-passing pathogenic organisms, and as complex auto-catalysts. When a cell is infected by most viruses, granules called *virus bodies* appear within the cytoplasm, or the nucleus, or within both. It is not clear whether they are aggregations of the virus or reaction bodies. Ludford (1942) has discussed the intra-nuclear inclusions and concluded that their presence alone is not proof of virus action.

Gresson and Zlotnik (1947) examined the Golgi substance of neurones from different regions of the brain and from the spinal cord of normal sheep and of animals infected with the virus of *louping-ill*. They found,

in the early stages of infection, an hypertrophy of the Golgi material and a reduction in the number of long Golgi filaments as compared with the normal neurone, especially in the basal part of the cell processes. In the later stages the Golgi material breaks up, but not to the same extent in all the regions of the nervous system examined, nor in all the cells of the same region (fig. 70).

Ludford and Findlay (1926) studied the epidermal cells of the chick infected with fowl-pox virus (fig. 71). Vacuoles appear in the cytoplasm, increase in size and number, and appear to fuse to form a body known as the *Bollinger body*. The larger vacuoles contain virus bodies. The Golgi material hypertrophies and looses its normal polarity. As it is often situated in close proximity to the virus bodies, it is suggested that it may be concerned with the formation or localization of a lipoidal substance which surrounds the vacuoles. The Golgi material finally breaks up. Hypertrophy and ultimate fragmentation of the Golgi bodies has been described in other types of cells infected with viruses.

CHAPTER XIX

AN INTRODUCTION TO CYTOLOGICAL TECHNIQUE

A COURSE of cytology should include some training in methods of examining living cells and of preparing permanent preparations. Many of the special methods are lengthy and require considerable experience if good results are to be obtained; they are, therefore, difficult to carry out in the limited time available for most classes. The student can, however, become proficient in the preparation of material by routine methods. If he is to make cytology his special study he must be prepared to learn patiently the special techniques involved, to obtain varying degrees of success with different types of tissue, and to persevere until he masters the methods appropriate to his special study. Only by following instructions carefully and through his own experience can he achieve success. The novice must learn to handle his microscope correctly and to interpret, according to our present knowledge, the structures seen in the optical field. In this chapter the general principles of cytological technique are briefly discussed and a few simple methods described. It is assumed that the student has had some previous experience of simple routine methods of fixing and staining tissues.

ANIMAL TISSUE

CHOICE OF MATERIAL.—The choice of material which is comparatively easy to prepare should be considered by the teacher before the class begins. In the spring and summer months a vast amount of invertebrate material, such as the ovotestis of *Helix* and the testes and ovaries of insect larvae and pupae, may be collected. The older oocytes of insects contain large quantities of yolk, and consequently are difficult to section and are unsuitable for the study of the cytoplasmic components. The testes of most larval insects are favourable for class work; the chromosomes are often small, but the stages of spermatogenesis can usually be followed without much difficulty. The testes of adult newts and grasshoppers make excellent preparations for the study of chromosomes. The nerve ganglia and testes of insects, when treated by an osmic method for the demonstration of the Golgi bodies, often give very good results.

During the autumn and winter the choice of material is limited. The testes of a small mammal, such as the mouse, may be used at any time of the year for the study of the spermatocyte divisions, and both testes and ovaries usually give good results when prepared by methods suitable for the preservation of the Golgi material and mitochondria. The mammalian pancreas is favourable tissue in which to observe the Golgi substance and secretion granules, and thin sections of the kidney, suitably fixed and stained, may be used to demonstrate the mitochondria. For the use of vital dyes, such as neutral red and Janus Green B, the gonads of insects and the testis and pancreas of the mouse are satisfactory.

FIXATION.—A piece of tissue taken from the body of an animal will undergo changes due to the action of bacteria, and changes of another kind quickly take place. Enzymes which synthesize proteins from the amino-acids resulting from the digestion of the food are present in all living cells. A cell which is no longer living becomes acid and the intracellular enzymes split the cell proteins into amino-acids; changes of this kind are spoken of as *autolysis*. A number of substances prevent autolysis from taking place, but a fixative must possess other properties as well. It is essential that all cytological material be placed in the fixing fluid as speedily as possible, and that the pieces of tissue be small, usually not more than about 2 mm. in thickness.

A good cytological fixative is a solution of chemical substances which prevents bacterial decay and autolysis, renders the substances of the cell insoluble and prevents them dissolving during subsequent treatment, and hardens the tissues, thus reducing shrinkage and distortion during preparation. Fixatives usually make the materials of the cell more easily stained and alter their refractive indices. Some fixatives precipitate the proteins of the cell and render them insoluble, and some preserve fats and other substances. Certain fixatives combine chemically with proteins and alter their structure. A fixing fluid containing two or three substances is usually employed for the routine histological examination of a tissue, but for detailed cytological work several fixatives should be used, including some which are suitable for the demonstration of particular cell structures.

WASHING.—After fixation the fixative must usually be washed out of the tissue. The method of washing varies with the nature of the fixative, but in most cases the tissue is left in running water for a certain time. A convenient method is to put the tissue into a solid watch glass covered with fine muslin, place in a large jar or other suitable container and leave under a gently running tap.

DEHYDRATION.—Water must be removed from tissue which is to be sectioned so that it may be impregnated with paraffin wax or some other medium. This is done by immersing in 30, 50, 70, 90 or 96 per cent and absolute alcohol. Small pieces of tissue for cytological work should be left for one hour in 30, 50, 70 and 90 per cent alcohol, and for half an hour

each in two changes of absolute alcohol. If 96 per cent alcohol is used instead of 90 per cent two changes of absolute alcohol are unnecessary, but the tissue must remain in the absolute alcohol for one hour. Long immersion in the higher grades of alcohol will harden tissues and make them difficult to section; therefore, if it is necessary to leave a piece of tissue in alcohol overnight, it is desirable to leave it in 50 or in 70 per cent alcohol.

EMBEDDING.—The tissue must be impregnated with paraffin wax, or with some other medium which will hold it together and allow it to be cut into thin sections by the microtome knife. Before placing the tissue in paraffin wax it is necessary to transfer it to an anhydrous substance which is miscible with both alcohol and paraffin wax. Xylene, cedar-wood oil, and benzene are commonly used, but xylene hardens tissues, and cedar-wood oil and benzene are to be preferred. As these substances render tissues translucent they are usually called clearing agents. Very delicate tissues should be placed for half an hour in a mixture of equal parts of the clearing agent and absolute alcohol, and then transferred to the clearing agent until they are translucent. The tissue is removed from the clearing agent and placed in hot paraffin wax (usually melting-point 57° C.) in an oven. Some workers recommend putting the tissue in a warm solution of equal parts of paraffin wax and the clearing agent for half an hour, and then transferring it to hot paraffin wax in the embedding oven. It is desirable to use two changes of pure paraffin wax and to allow the tissue to remain in each for about an hour. The time necessary for the paraffin to infiltrate depends upon the size of the piece of material, and also, to a certain extent, upon the type of tissue. The tissue and wax is finally removed from the oven and a paraffin block prepared in the usual way.

SECTION CUTTING, STAINING AND MOUNTING.—For the demonstration of the cytoplasmic components, sections should be cut at 3 μ (or less) to 8 μ in thickness, but for the examination of chromosomes it is often an advantage to cut sections very much thicker.

The principles of staining cannot be discussed here. Before staining, the sections are placed on a slide and dried in the usual manner. The paraffin is removed by xylene, and the sections are then placed successively in absolute and the lower grades of alcohol. In most cases the sections are dehydrated after staining, cleared in xylene and mounted in Canada balsam.

METHODS OF FIXING AND STAINING.—The present account is designed as a brief introduction for the student who has already made some simple histological preparations, and who now intends to prepare sections and smears suitable for cytological work. The student who wishes to make detailed cytological studies will have to determine the most suitable methods for his own particular purpose, vary the times of staining when

necessary, and perhaps use methods of embedding other than the one described in this chapter. The fixatives given in this section are only a few of the many known to cytologists and will not yield equally satisfactory results with every type of animal tissue. They are given in order that the student may gain experience in handling tissue and in making permanent preparations. Further details of these methods of fixing and staining and of other techniques will be found in the references. It is desirable to begin with a general fixative, such as Bouin's or Zenker's fluid, and then to try special methods for chromosomes, Golgi material and mitochondria. Good preparations of mitochondria are the most difficult to obtain.

Bouin's Picro-Formal

Picric acid, saturated aqueous solution	75 c.c.
Formal	25 c.c.
Acetic acid	5 c.c.

Fix for 16-24 hours according to the size of the piece of tissue. Wash in several changes of 50 per cent alcohol until most of the picric acid is removed. Upgrade to absolute alcohol and clear in cedar-wood oil or benzene. Sections usually stain well with Heidenhain's iron haematoxylin and other routine stains.

For general purposes Bouin's picro-formal usually gives good results. Tissues may be left in the fixative for long periods without damage, and it is, therefore, a valuable fixative for field work. It may be used for almost any tissue. Modifications of Bouin's fluid exist.

Zenker's Fluid

Potassium dichromate	2·5 grms.
Mercuric chloride	5 grms.
Distilled water	100 c.c.

Immediately before use add 5 c.c. of glacial acetic acid to 20 c.c. of the mixture.

Fix for 24 hours or overnight. Wash for several hours or overnight in running water. Material fixed in fluids containing mercuric chloride should be treated with iodine to remove precipitate. This may be done by immersing sections for 2 minutes in 0·5 per cent solution of iodine in 70 per cent alcohol; rinse in 70 per cent alcohol. To remove yellow stain due to iodine place the slide in 5 per cent sodium thiosulphate for one minute. Wash in running water for a few minutes.

Zenker's fluid may be used for routine work. Haematoxylin and other common stains may be used.

Flemming's Fluid

1% chromic acid	15 c.c.
2% osmium tetroxide	4 c.c.
Glacial acetic acid	Few drops

Fix for 24 hours. Wash for 2 hours or longer in running water. Stain in Heidenhain's iron haematoxylin. Mordant in 3 per cent iron alum for several hours and stain in haematoxylin overnight.

The pieces of tissue must be small as penetration is uneven. Good general fixative for chromosomes. If used without acetic acid the mitochondria are preserved and so is the Golgi material of male germ-cells and sometimes that of other tissues. For the study of mitochondria sections should be thin—2 μ–5 μ in thickness. Sections may be stained with iron haematoxylin or with acid fuchsin.

METHODS FOR CHROMOSOMES

The student should begin with a simple fixing fluid such as Bouin's picro-formal or Flemming's fluid. If Flemming's fluid is used add 0·5 c.c. of glacial acetic acid to 19 c.c. of the stock solution. Sections fixed with picro-formal may be stained with iron haematoxylin. Mordant in iron alum for 15 minutes to 2 hours; stain in haematoxylin for half an hour to 3 hours. Material fixed in Flemming may be stained with haematoxylin as above, or with crystal violet. Stain for 3–10 minutes with 1 per cent aqueous crystal violet which has been boiled and filtered. Rinse with distilled water. Transfer for 30–45 seconds to 80 per cent alcohol containing 1 per cent iodine in 1 per cent solution of potassium iodide. Rinse for 2 seconds in 95 per cent alcohol and 2–5 seconds in absolute alcohol. Differentiate in clove oil. Clear in three changes of xylene for 15 minutes. Sections for the demonstration of the chromosomes should be at least 10 μ in thickness.

SMEARS.—Make a smear of an insect testis on a glass slide. Place the slide for half a minute in a jar provided with a glass stopper and containing a few c.c. of osmium tetroxide. Remove the slide. Place it in a shallow vessel and pour in 1 per cent chromic acid. Leave for one hour. Wash for one hour in running water and stain with crystal violet or with iron haematoxylin.

SCHNEIDER'S ACETO-CARMINE.—For salivary gland chromosomes of the Diptera. The salivary glands of the larvae of *Chironomus* are suitable.

Dissolve 10 grms. of carmine in 100 c.c. of hot 45 per cent aqueous glacial acetic acid; bring up to boiling-point; cool and filter. Dissect out the salivary glands by cutting the body of the larva in two a little behind the head. Press the posterior part of the body and identify the salivary glands under a low power binocular. Remove the other tissues. Add a little aceto-carmine and leave for 10–20 minutes. Cover preparation with a cover-glass. Remove excess stain with filter paper. Apply gentle pressure to cover-glass, being careful not to allow the cover-glass to move. Seal cover-glass with vaseline or paraffin wax.

PERMANENT ACETO-CARMINE PREPARATIONS.—The salivary glands are stained as above. It may be desirable to leave in the aceto-carmine for several hours. Cover with cover-glass. Place in dish containing alcohol fumes prepared by lining dish with filter paper saturated with 95 per cent alcohol. Leave for 1–24 hours. Place overnight in jar containing 95 per cent alcohol.

The cover-glass is removed carefully and the preparation is mounted in euparal. It is advisable to treat the slides with egg albumen so that the tissue sticks to the slide.

METHODS FOR THE GOLGI MATERIAL AND MITOCHONDRIA

Altmann's Fluid

5% potassium dichromate. . .	1 vol.
2% osmium textroxide . . .	1 vol.

Fix for 24 hours. Wash overnight in running water. Mount sections on slide, dry and stain for one minute over flame with solution of 5–7 grms. of acid fuchsin in 100 c.c. of aniline oil water. The aniline oil water is prepared by adding the oil to distilled water until no more dissolves; shake from time to time and then filter. Cool sections and differentiate over flame with a saturated alcoholic solution of picric acid diluted with two volumes of distilled water. Rinse quickly in 90 per cent alcohol, place in absolute alcohol and clear in xylene. If the sections are cut at 2 μ or 3 μ in thickness, this method is excellent for the demonstration of mitochondria. It is not suitable for chromosomes. Baker recommends the addition of 0·7 per cent sodium chloride, and for mitochondria post-chroming for about 48 hours in a saturated solution of potassium dichromate at 37° C.

Kolatchev's Fluid

6% potassium dichromate . . .	7 c.c.
1% chromic acid	7 c.c.
2% osmium tetroxide . . .	7 c.c.

For Golgi material. Fix for 24 hours. Wash overnight in running water. Keep in 1–2 per cent osmium tetroxide for 3–7 days at 30–50° C. 4–5 days usually give good results with most tissues. Very small pieces of the tissue should be removed from time to time, washed and mounted in glycerin. The cells can be separated by pressure on the cover-glass and examined under the microscope to determine when the correct degree of impregnation is reached. Wash in running water for several hours. Upgrade to absolute alcohol and clear in cedar-wood oil. Sections may be counterstained in neutral red or mounted unstained. Stain for about half a minute in solution of 1 grm. of neutral red in 1000 c.c. of distilled water to which is added 2 c.c. of 1 per cent solution of glacial acetic acid.

Rinse in distilled water, differentiate in absolute alcohol, and clear in xylene.

In Kolatchev preparations the Golgi material is black. The mitochondria are usually unstained, or are yellowish; if they are blackened the colour may sometimes be extracted by placing the slide in turpentine after the wax is removed by xylene.

Sometimes the colour is extracted from the Golgi material if the sections are mounted in Canada balsam; it is often desirable, therefore, to mount in euparal.

Aoyama's Fluid

Cadmium chloride	1 grm.
Neutral formal	15 c.c.
Distilled water	85 c.c.

For Golgi material. Fix for 3–4 hours. Rinse in two changes of distilled water. Keep in 1·5 per cent silver nitrate for 10–15 hours in the dark at 22° C. Rinse in two changes of distilled water. Transfer to reducing solution for 5–10 hours at 22° C.

Hydroquinone	1 grm.
Neutral formal	15 c.c.
Anhydrous sodium sulphite	0·15 grm.
Distilled water	85 c.c.

Wash in running water for about half an hour. Upgrade to absolute alcohol and embed. The Golgi material is black and the mitochondria when shown are golden brown.

Aoyama's method frequently gives very good results, but silver methods are uncertain. The correct time to leave the material in silver nitrate solution should be determined for different tissues. Sections may be stained in Ehrlich's haematoxylin for 10–15 minutes. Some of the sections should be toned with gold chloride. Dissolve the wax from the sections and bring down to distilled water. Place the slide for 15 minutes in 0·1 per cent solution of gold chloride. Rinse in distilled water. Place the slide for 5 minutes in 5 per cent sodium thiosulphate. Wash and dehydrate. The sections may be stained in Ehrlich's haematoxylin or mounted unstained.

SUPRAVITAL STAINS

Mitochondria and pre-existing granules in living cells may be stained with certain dyes. Janus Green B and neutral red are commonly used; neutral red is the least toxic and is more likely to yield good results in the hands of the novice.

JANUS GREEN.—For staining mitochondria. Small pieces of living tissue are teased out in physiological saline solution and are then trans-

ferred to a 1 : 20,000 to 1 : 50,000 solution of Janus Green B in physiological saline. After standing for 10–20 minutes the preparation is covered with a cover-glass and examined under an immersion lens. If tissues from warm-blooded animals are used, the preparations should be kept in an oven at body temperature until ready for examination. In successful preparations the mitochondria are stained by the dye, but usually lose their colour rapidly.

NEUTRAL RED. — Stains pre-existing granules and may also be segregated into vacuoles. Treat the tissue in the same way as for Janus Green, using dilutions of 1 : 20,000 to 1 : 50,000 or less. Baker (1944) recommends a method of using neutral red to demonstrate the " Golgi element ". The ovotestis of *Helix* or the spermatocytes of the mouse are suitable for class work. When the tissue is no longer living the nucleus and the cytoplasm stain diffusely.

With both Janus Green and neutral red it is advisable to work in a darkened room and to open the iris diaphragm until the field is suitably illuminated to show the stained bodies. Good quality stains are essential.

PLANT TISSUES

Some of the fixing fluids used for preserving plant tissues for histological examination are similar to those employed for animal material. The fixatives commonly used, however, have great powers of penetration and preserve the grosser cell structures rather than the finer details of the nucleus and the cytoplasm. Many different methods of fixation and of subsequent treatment are recommended for the preservation of various cell structures and substances, and it is necessary to use special techniques for different groups of plants. As many of the methods are limited in application, it is not possible to describe them here. The student desirous of preparing plant tissues for general and special purposes should consult a standard work on technique.

CHROMOSOMES.—Fixatives such as Flemming's fluid and the aceto carmine methods are satisfactory (pp. 160-162). Modifications of Flemming's method are recommended by some workers.

CYTOPLASMIC COMPONENTS.—The mitochondria of plant cells are preserved by the methods used for their study in animal tissues. They may also be studied by fixing tissue for 48 hours in neutral formalin and subsequently staining in Heidenhain's haematoxylin. Osmiophilic platelets are revealed by osmic techniques and are said by some workers to be homologous to the Golgi material of the animal cell. Plastids are also preserved by osmic methods.

GLOSSARY

The terms included in the following list are those used in the text. Terms likely to be familiar to students of botany and zoology are, for the most part, omitted. The meanings given are not necessarily the original ones, but are those which conform with modern usage.

Accessory body: A body which originates within the Golgi zone of spermatocytes and spermatids. An accessory body is included in the neck of the spermatozoon.

Acrosome: A structure present on the head of the spermatozoon.

Agamospermy: Synonym for apogamy.

Allelomorph: One of a pair of dissimilar hereditary factors or genes.

Allopolyploid: An organism with more than two haploid sets of chromosomes in its nuclei which have been derived by hybridization from different species. See *Polyploid.*

Alternation of generations: The alternation of two phases, a haploid and a diploid, in the life cycle of a plant.

Amitosis: Direct nuclear division without the formation of a spindle or the separation of daughter chromosomes.

Amphiaster: A spindle provided with two asters.

Anaphase: The stage of mitosis which follows the metaphase. During the anaphase the chromosomes move from the equatorial region of the spindle towards the poles.

Anastral spindle: A spindle which is not provided with asters.

Androspore: The microspore or pollen grain of Angiosperms.

Anisogamy: A form of heterogamy in which the gametes show only slight differences in size and behaviour.

Antheridium: Male reproductive organ of the Thallophyta, Bryophyta and Pteridophyta.

Apogamy: A form of apomixis in which the sporophyte develops from cells of the gametophyte other than the egg-cell.

Apomixis: Reproduction with the outward appearance of sexual reproduction but without gametic fusion and often the omission of meiosis.

Apospory: Formation of a gametophyte from extra-archesporial tissue such as unspecialized cells of the nucellus or integument.

Archegonium: Female reproductive organ of the Thallophyta, Bryophyta, Pteridophyta and some Spermaphyta (Gymnosperms).

Archoplasm: The cytoplasm which surrounds the centrosome during the interphase.

Archoplasmic granule: See *Proacrosomic granule.*

Archoplasmic vacuole: A vacuole which surrounds a proacrosomic granule or the proacrosome.

Aster: The radiating structure which surrounds a centrosome during mitosis.

Autopolyploid: An organism with more than two haploid sets of chromosomes in its nuclei which have arisen by multiplication in an individual which is not a hybrid.

Autosome: An ordinary chromosome as distinguished from a sex chromosome.

Bivalent: Two homologous chromosomes which have paired during meiosis.

Blepharoplast: A small body at the base of a cilium or a flagellum.

Brachymeiosis: The third division in the ascus of the Ascomycetes which, like the first, is believed to involve a reduction in the chromosome number in those species in which the primary ascus nucleus appears to be tetraploid.

Cell-plate: See *Phragmoplast.*

Central body: A term sometimes used to describe the centrosome and centriole.

Centriole: A small body which is usually surrounded by a centrosome.

Centromere: A special region of a chromosome by which the latter is attached to the spindle during mitosis.

Centrosome: A self-propagating body which contains one or more centrioles; during mitosis in many organisms a centrosome is situated at each pole of the spindle.

Chiasma: A chiasma is formed by two of the four chromatids of a bivalent breaking at the same level at the end of the pachytene stage and then joining diagonally so that there is an exchange of parts. There may be several chiasmata in each bivalent.

Chondriosomes: A term used for all forms of mitochondria—granules, rods, etc.

Chromatid: A longitudinal half of a chromosome at mitosis.

Chromatin: A term used for the material of chromosomes which stains deeply during mitosis. The deeply stained chromatin granules and masses of the non-dividing nucleus are artifacts.

Chromomeres: Minute bodies arranged in linear series on a chromosome. They are probably identical with genes.

Chromosomes: Structures present in definite numbers in the nuclei of plants and animals. They carry the hereditary factors or genes.

Crossing-over: An exchange of corresponding parts between two of the four chromatids at the end of the pachytene stage. See *Chiasma.*

Cytokinesis: The division of the cytoplasm which follows mitosis and meiosis.

Cytoplasm: The extra-nuclear protoplasm.

Deletion: The loss of a part of a chromosome due to the chromosome breaking at one or two points. Deletions are usually intercalary.

Deutoplasm: Yolk and nutritive material of the ovum.

Diakinesis: Last stage in the prophase of the first meiotic division.

Dikaryon: The paired association of male and female nuclei without fusion in the mycelium of certain Fungi.

Diplohaplonts: Algae with two well-marked, though morphologically identical, generations in which the haploid phase bears the gametes, the diploid the asexual spores, and meiosis occurs during zoosporogenesis.

Diploid: The somatic number of chromosomes; it consists of two haploid sets. A diploid organism has a diploid group of chromosomes in each of its somatic nuclei.

Diplonts: Plants, particularly Algae, in which the normal vegetative phase is diploid and meiosis occurs during gametogenesis.

Diplospory: Form of apomixis in which the embryo is developed either from the egg-cell (parthenogenesis) or from some other gametophytic cell (apogamy).

Diplotene: Stage in the prophase of the first meiotic division which follows the pachytene stage.

Duplication: The occurrence of a portion or portions of a chromosome more than once.

Embryo-sac: Female gametophyte of the Angiosperms.

Female pronucleus: The egg-nucleus after the completion of the maturation divisions.

Gamete: A ripe germ-cell.

Gametocyte: Cell which, by division, produces gametes.

Gametogenesis: The series of changes by which the primitive germ-cells are transformed into the gametes.

Gametophyte: The haploid stage which produces the gametes in the life-history of a plant showing an alternation of generations.

Genes: Hereditary factors located on the chromosomes. Some workers believe that other hereditary factors are present in the cytoplasm.

Genotype: The hereditary constitution of an organism.

Germinal vesicle: The large vesicular nucleus of a primary oocyte.

Golgi apparatus: A component of the cytoplasm of the animal cell. It may be visible as a net-like structure or group of bodies at one pole of the nucleus, or it may be distributed as separate bodies through the cell. The term Golgi apparatus is often restricted to the localized condition. See *Golgi material.*

Golgi body: A body composed of Golgi material.

Golgi element: Synonym for Golgi body. Used by Baker for the Golgi material and associated fluid vacuoles.

Golgi material: Some cytologists believe that the Golgi "network" consists of separate Golgi bodies which lie close together so as to give the appearance of a network. As the material assumes different forms in different phases of the cell, the term Golgi material or Golgi substance is preferred to Golgi apparatus. See *Golgi apparatus.*

Golgi remnant: Part of the Golgi material of the spermatid which is eliminated with the residual cytoplasm.

Golgi substance: See *Golgi material.*

Gonad: The gland in which the gametes are produced; the ovary or testis.

Gonomery: A condition in which the paternal and maternal chromosomes appear in separate groups during the cleavage stages of some organisms.

Gynospore: The megaspore or embryo-sac of Angiosperms.

Haploid: The reduced or gametic number of chromosomes; a single group containing one of each kind of chromosome.

Haplonts: A group of Algae in which only the zygote is diploid.

Heterogametic: Producing gametes of more than one kind; applied to gametes which differ in respect to the sex chromosomes.

Heterogamy: Production of distinct male and female gametes.

Heteropycnosis: The property which certain chromosomes, or parts of a chromosome, possess of condensing at a different rate from the other chromosomes, or parts of a chromosome, in the nucleus.

Heterospory: Production of two types of spores, microspores and megaspores.

Heterothallism: The existence of two strains of certain Fungi, which when grown apart produce only sporangia, but when grown in contact produce zygospores.

Heterozygous: An organism is said to be heterozygous for a given allelomorphic gene if this gene is present on one member of a pair of chromosomes and its allelomorph is present on the other member of the pair.

Homogametic: Producing gametes which are all alike; applied to gametes which are alike as regards their sex chromosomes.

Homologous chromosomes: The two members in a diploid set which constitute a pair of similar chromosomes.

Homospory: Production of only one type of spore from which bisexual gametophytes arise.

Homothallism: Production of zygospores upon a thallus derived from the culture of a single spore.

Homozygous: An organism is said to be homozygous for a given allelomorphic gene if that gene is present on both members of a pair of chromosomes.

Hyaloplasm: Clear non-granular protoplasm.

Interference: The process by which the occurrence of a chiasma reduces the probability of another taking place for some distance on either side of the chiasma.

Interphase: The stage in the history of the nucleus between the end of one mitosis and the beginning of the next.

Inversion: A reversal in the position of a part of a chromosome in comparison with the normal sequence.

Isogamy: Fusion of morphologically identical gametes.

Karyogamy: Union of the gamete-nuclei.

Karyokinesis: Synonym for mitosis.

Leptotene stage: Early prophase of the first meiotic division before the chromosomes have paired.

Male pronucleus: The sperm-nucleus during fertilization.

Maturation: The final stages in the development of the germ-cells.

Megaspore: Spore which produces a gametophyte bearing only female gametes.

Meiosis: Two modified mitoses of the germ-cells, during which the chromosomes divide only once.

Metaphase: Stage of mitosis which follows the prophase.

Metaplasm: Inclusions of the cytoplasm, such as yolk, fat droplets, etc.

Microspore: Spore which produces a gametophyte bearing only male gametes.

Mitochondria: Components of the cytoplasm. They may be in the form of granules, rods, filaments or spheres.

Mitosis: Division of the nucleus to form daughter nuclei. It involves a series of nuclear and cytoplasmic changes. See *Cytokinesis*.

Nucellar embryony: Formation of a new sporophytic generation directly from a cell, or cells, of the nucellus without the intervention of a gametophytic stage.

Nuclear-ring: Ring-shaped structure which surrounds the head of the late spermatid and sperm of certain mammals.

Oocyte: An egg-cell prior to the completion of maturation.

Oogenesis: Development of an egg from its origin from an oogonium to the formation of the ovum.

Oogonium: Cell from which oocytes are derived.

Ovum: An egg-cell which has undergone a process of maturation.

Pachytene stage: The part of the prophase of the first meiotic division when pairing of the chromosomes is complete; it follows the zygotene stage.

Parthenogenesis: Development of a female gamete into an embryo without fertilization.

Phragmoplast: Barrel-shaped structure formed by the widening of the spindle in plant cells; the cell-plate is formed within the phragmoplast.

Plasmodesma: Protoplasmic connections between neighbouring cells.

Plastids: Cytoplasmic bodies of plant cells; intimately concerned with carbohydrate metabolism.

Polar body: Minute cell which separates from the egg during the maturation divisions.

Polocyte: Synonym for polar body.

Polyploid: Cells or organisms which possess more than two haploid sets of chromosomes in their nuclei.

Polysomy: A condition in which one or more chromosomes, but not the whole haploid set, are present more than twice in the complete chromosome complement.

Polyspermy: Penetration of the egg by more than one sperm.

Post-nuclear cap: Structure which invests the posterior part of the nucleus of the sperm of some animals.

Proacrosome: Structure present in the spermatid from which is formed, in whole or in part, the acrosome.

Proacrosomic granule: Granules present in the Golgi zone of the spermatocyte and young spermatid; they fuse to form the proacrosome.

Prometaphase: Final stage of the prophase after the dissolution of the nuclear membrane.

Prophase: The first stage of mitosis.

Prothallus: Gametophyte of the Pteridophyta.

Protoplasm: The living substance of the cell.

Protoplasmic bead: Protoplasmic structure present on the anterior part of the middle-piece of the spermatozoon of mammals.

Spermateleosis: The metamorphosis of the spermatid into the spermatozoon.

Spermatid: Cell which is formed by division from a spermatocyte and is metamorphosed into a spermatozoon.

Spermatocyte: Cell derived from a spermatogonium and which gives rise to spermatids.

Spermatogenesis: Development of the male germ-cells from their origin from spermatogonia to the formation of the spermatozoa.

Spermatogonium: Cell derived from primitive germ-cells and which gives rise to spermatocytes.

Spindle: Structure formed during the prophase of mitosis. See *Spindle element* and *Spindle fibre.*

Spindle attachment: See *Centromere.*

Spindle element: Elements of which the spindle is probably composed.

Spindle fibre: Fibres seen in fixed preparations of the spindle; the continuous spindle fibres stretch from pole to pole, and the half-spindle fibres stretch from one pole to the equator of the spindle.

Sporophyte: The diploid generation producing asexual spores in the life-history of a plant showing an alternation of generations.

Syngamy: Union of the gametes during fertilization.

Telophase: Final stage of mitosis during which the daughter nuclei are formed and cytokinesis takes place.

Terminalization: The movement of chiasmata from their original positions towards the ends of a bivalent.

Tetrad: The four chromatids of which each bivalent is composed during the later stages of the prophase of the first meiotic division.

Translocation: The transfer of a part of a chromosome, by means other than normal crossing-over, to another chromosome which may not be homologous; translocation may or may not involve a reciprocal exchange of parts.

Vacuolar System: System of vacuoles in the cytoplasm of plant cells, each of which is bounded by a semi-permeable membrane and contains a watery solution of various metabolic products.

Zygote: Cell formed by the union of the gametes.

Zygotene stage: Stage of the prophase of the first meiotic division during which pairing of the homologous chromosomes takes place; it follows the leptotene stage.

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